HANDBOOK OF PROTOCTISTA

THE STRUCTURE, CULTIVATION, HABITATS AND LIFE HISTORIES OF THE EUKARYOTIC MICROORGANISMS AND THEIR DESCENDANTS EXCLUSIVE OF ANIMALS, PLANTS AND FUNGI

A guide to the algae, ciliates, foraminifera, sporozoa, water molds, slime molds and the other protoctists

EDITORS:

Lynn Margulis
University of Massachusetts - Amherst, MA

John O. Corliss
University of Maryland - College Park, MD

Michael Melkonian
Universität zu Köln, Botanisches Institut
Federal Republic of Germany

David J. Chapman
University of California - Los Angeles, CA

EDITORIAL COORDINATOR:

Heather I. McKhann
University of California - Los Angeles, CA

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Phylum Zoomastigina
Class Kinetoplastida

Keith Vickerman

INTRODUCTION

General Characteristics

The Kinetoplastida constitute an order* of small colorless flagellates with one or two flagella and massed mitochondrial DNA that forms a stainable structure—the kinetoplast—within the single mitochondrion. The kinetoplast usually lies close to the kinetosomes of the flagella (eukinetoplastic condition), but in some genera several kinetoplasts may be present in the mitochondrion (polykinetoplasty) or the kinetoplast DNA may be irregularly spread throughout the mitochondrion (pankinetoplasty). In a few mutants stainable kinetoplast DNA may be absent (dyskinetoplasty). The size, shape and position in the body of the kinetoplast are of taxonomic and ontogenetic significance. This order includes the pathogenic trypanosomes and leishmanias, so the kinetoplastids are of major medical, veterinary, and economic importance.

Sixteen genera may be confidently assigned to the order and about 550 species have been described. The order is divided into two unifamilial suborders, the Bodonina and the Trypanosomatina.

Members of the Bodonina (family Bodonidae) have two heterodynamic flagella—one directed anteriorly and locomotory, the other directed posteriorly and recurrent or trailing (Fig. 1). The suborder includes many free-living forms. Bodonines are usually phagotrophic, ingesting food through a cytopharynx whose cytostome is bordered by prominent lips often drawn out into a projecting rostrum (Fig. 1b,e,g). The majority are eukinetoplastic, but some are polykinetoplastic (Fig. 1e,f,g) or pankinetoplastic (Fig. 1i). The diagnostic characteristics of the bodonine genera are summarized in Table 1.

Members of the suborder Trypanosomatina (family Trypanosomatidae)—often referred to as hemoflagellates—are uniflagellate, entirely eukinetoplastic, and invariably parasitic (the only described free-living genus, *Proleptomonas,* is now known not to be a kinetoplastid (Vickerman and Le Ray, 1977). During their life cycles, the position of the kinetoplast-kinetosome-flagellar pocket complex may shift in relation to the nucleus and body extremities, and the flagellum may change in length. Thus, the following life cycle stages are recognized (Fig. 2): *amastigote*—round to oval body, flagellum short, not emerging from pocket; *promastigote*—kinetoplast close to anterior end of elongate body, flagellum emerging anteriorly and unattached; *opisthomastigote*—similar, but kinetoplast is postnuclear and flagellar pocket forms long canal to anterior end of body; *choanomastigote*—body pyriform, kinetoplast just in front of nucleus, flagellum emerges anteriorly; *epimastigote*—prenuclear kinetoplast, flagellum emerges from pocket partway along body and is attached to body along its anterior portion; *trypomastigote*—similar, but kinetoplast and flagellar pocket are postnuclear. The term spheromastigote is sometimes used for a rounded body with an emerging flagellum attached to it. The different genera are characterized according to which of these morphological stages are present in the life cycle and whether the cycle is monogenetic (monoxenous) or digenetic (heteroxenous) (Table 2), i.e., involves one host or alternates between two different animal hosts, usually a vertebrate and an invertebrate.

The number of species cited for trypanosomatids in Table 2 is approximate, as new species are constantly being described; moreover, many named species are morphologically identical and can be distinguished only on the basis of host specificity, host range, or the clinical features of the disease which they cause. Such taxa are considered by some as subspecies, by

*Refer to footnote, p. 194.

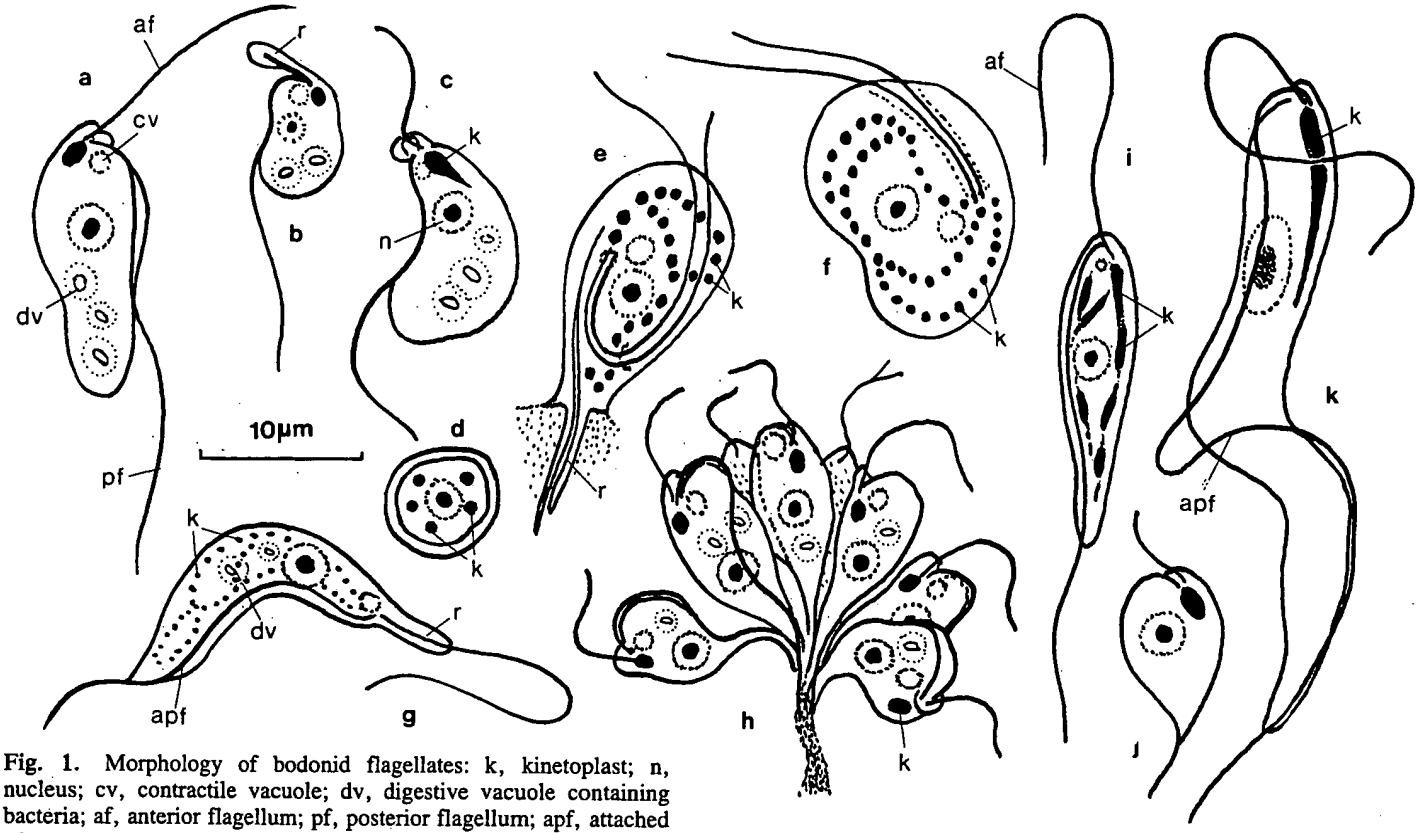

Fig. 1. Morphology of bodonid flagellates: k, kinetoplast; n, nucleus; cv, contractile vacuole; dv, digestive vacuole containing bacteria; af, anterior flagellum; pf, posterior flagellum; apf, attached pf; r, rostrum; EUK, eukinetoplastic; PLK, polykinetoplastic; PNK, pankinetoplastic. a, *Bodo caudatus* (EUK); b, *Rhynchomonas nasuta* (EUK); c, *Procryptobia glutinosa* (EUK); d, *P. glutinosa* cyst (PLK); e, *Icthyobodo necator* (PLK) attached phase on fish skin; f, *I. necator* (PLK) migratory phase; g, *Dimastigella trypaniformis* (PLK); h, *Cephalothamnium cyclopum* (EUK) colony with secreted stalk; i, *Cryptobia vaginalis* from leech (PNK phase); j, *Cryptobla vaginalis* (EUK phase); k, *C.* (*Trypanoplasma*) *keysselitzi* (EUK) from tench. (After Vickerman and Preston, 1976).

others as "biological races." The species recognition problem is most acute in the practically important genera *Trypanosoma* and *Leishmania*, and for this reason Hoare (1972) created sections and subgenera within the mammal parasitizing *Trypanosoma* species. Table 3 summarizes this subgeneric classification (see also *Classification Schemes*, p. 000). Subgeneric classifications have not been proposed for other genera.

Occurrence

Many of the bodonids are free-living organisms. Epizoic bodonids live on the gills and skins of fish and a few other organisms. Some bodonids are parasites of fish and other aquatic organisms (see Table 1). All trypanosomatids are parasitic, and their host range is indicated in Table 2. Species of *Bodo*, *Rhynchomonas*, and all trypanosomatid genera except *Rhynchoidomonas*, may be obtained as cultures from the American Type Culture Collection.

Literature

Accounts of the pathogenic Trypanosomatidae loom large in texts on tropical medicine and veterinary medicine as well as in frequent papers in journals dealing with these subjects. An excellent account of the organisms now included in this order, with exhaustive lists of described species of Trypanosomatidae, are given in Wenyon's textbook (1926). The first attempt to monograph burgeoning knowledge of trypanosomes was that of Laveran and Mesnil (1904, 1912). Comprehensive separate reviews of the Bodonina and the Trypanosomatina were published in Grassé (1952), but the Kinetoplastida were not monographed as an order until the mid-1970s (Lumsden and Evans, 1976, 1979). Outstanding recent work dealing with more specialized aspects of the order include Mulligan (1970), Hoare (1972), The Ciba Symposium (1974), Losos and Chouinard (1979), articles in the series edited by Levandowsky and Hutner (1979–1981), and Volume I of Kreier (1977). Reviews on parasitic kinetoplastids appear regularly in *Advances in Parasitology* and *The Tropical Diseases Bulletin*, published by Academic Press. In addition to the usual protozoological and parasitological journals, recent work on kinetoplast DNA and trypanosome antigenic variation is to be found in journals of molecular biology such as *Nucleic Acids Research*, *Cell*, and *EMBO Journal*.

History of Knowledge

The kinetoplast was identified as the first cytoplasmic structure to contain DNA (i.e., Feulgen-positive) by Bresslau and Scremin (1924). This was 80 years after the first description of these blood parasites or hemoflagellates, now known to possess a kinetoplast (Valentin, 1841). The order Kinetoplastida was created by Honigberg (1963) by uniting the two families of kinetoplast-bearing flagellates (Bodonidae and Trypanosoma-

TABLE 1. Principal genera of Kinetoplastida Bodonina

Genus	*Diagnostic Characters*[a]	*Number of Described Species*	*Feeding*	*Habitat/ Life Cycle*
Bodo Ehrenberg 1830	Eukinetoplastic trophozoite; PF free from body; rostrum small. Cysts formed in most species; polykinetoplastic cyst in some.	c.30	Phagotrophic, bactivorous	Free-living (fresh or sea water, soil); commonly coprozoic; can colonize fleeting habitats (dew, spittle bug exudate).
Rhynchomonas Klebs 1893	Eukinetoplastic trophozoite; PF mostly free from body, acts as "skid"; short AF attached to long proboscis formed from rostrum. Cysts undescribed.	3	Phagotrophic, bactivorous	Free-living (fresh water, marine planktonic, soil); coprozoic.
Ichthyobodo Pinto 1928 (=*Costia* Leclerque 1890)	Polykinetoplastic; trophozoite attached to host by elongate rostrum; free swimming dispersive stage lacks rostrum; PF free from body. No cysts.	1	Phagotrophic ingesting cytoplasm of host cell. (histophagic)	Biphasic life cycle; trophozoite ectoparasitic on fish and urodeles; alternates with free-swimming dispersive phase. Pathogenic in young fish.
Procryptobia Vickerman 1979	Eukinetoplastic trophozoite; PF attached to body over greater length, acts as "skid." Cysts sometimes polykinetoplastic.	3	Phagotrophic, bactivorous	Free-living; commonly coprozoic.
Dimastigella Sandon 1928	Polykinetoplastic trophozoite and cyst; PF attached along elongate body acts as "skid." Long rostrum alongside AF.	1	Phagotrophic, bactivorous	Free-living in soil and coprozoic.
Cephalothamnium Stein 1878	Eukinetoplastic; PF attached along length of body; colonial flagellates united in clusters by posterior extremities, PFs inserted in communal secreted stalk.	1	Phagotrophic, bactivorous	Sedentary and epizoic on freshwater copepods.
Cryptobia Leidy 1846 (=*Trypanoplasma* Laveran and Mesnil 1901)	Eukinetoplastic or pankinetoplastic trophozoites; PF attached along length of body; cysts unknown. In digenetic trypanoplasms (sometimes separated as genus *Trypanoplasma*) PF forms "undulating membrane."	c.60	Osmotrophic	Parasitic or endocommensal in gut or reproductive system of invertebrates; gills and gut of fish; blood of fish with cyclical transmission by leeches. Occasionally pathogenic in fish.

[a]Abbreviations: AF—anterior flagellum; PF—posterior flagellum.

TABLE 2. Principal genera of Kinetoplastida Trypanosomatina

Genus	Diagnostic Characters[a]	Number of Described Species	Hosts and Practical Significance
Leptomonas Kent 1880	Monogenetic; *promastigotes* and cysts only in life cycle.	c.70	Mainly insects (Hemiptera, Diptera, Hymenoptera, Blattoidea, Lepidoptera, Siphonaptera, Anoplura), rarely other invertebrates and protozoa. Some so called "lizard leishmanias" may belong in this genus. Nonpathogenic.
Herpetomonas Kent 1880	Monogenetic, promastigotes and *opisthomastigotes* in life cycle.	c.20	Diptera, possible other insect orders. Nonpathogenic.
Crithidia Léger 1902	Monogenetic; *choanomastigotes* only.	c.30	Diptera, Hemiptera, Trichoptera; Hymenoptera. Nonpathogenic.
Blastocrithidia	Monogenetic; *epimastigotes* and cysts only	c.30	Diptera, Hemiptera, Siphonaptera and ixodid ticks. Possibly pathogenic in some species.
Rhynchoidomonas Patton 1910	Monogenetic; *trypomastigote* stage only (but genus poorly known).	3	Diptera, Lepidoptera. Nonpathogenic.
Phytomonas Donovan 1909	Digenetic; *promastigotes*.	c.20	Plants (Euphorbiaceae, Asclepiadaceae, Moraceae, Palmae, mainly) *and* phytophagous Hemiptera. Pathogenic species cause hartrot in oil and coconut palms, and wilt disease in coffee plant.
Leishmania Ross 1903	Digenetic; intracellular *amastigotes* (mammal) and promastigotes (vector).	c.20	Mammals (Primates, Rodentia, Edentata, Hyracoidea, Carnivora, Marsupialia) and Diptera (Phlebotominae). Pathogenic species in man cause dermal, mucocutaneous, and visceral leishmaniasis (see Table 5).
Endotrypanum Mesnil and Brimont 1908	Digenetic; intraaerythrocytic trypomastigotes and epimastigotes (in mammal); promastigotes and amastigotes (in vector).	2	Edentata (sloths) and Diptera (Phlebotominae, genus *Lutzomyia*). Nonpathogenic.
Trypanosoma Gruby 1843	Digenetic; *trypomastigotes* (and more rarely epimastigotes or intracellular amastigotes) in vertebrate, epimastigotes (rarely promastigotes, amastigotes) in vector.	c.300	Vertebrates (all classes) and Hirudinea or Arthopoda (insects, mites). Pathogenic species cause sleeping sickness and Chagas' disease in man, nagana and related diseases in domestic animals (see Tables 3 and 4).

[a]The morphological type characteristic of each genus is given in italics. In *Endotrypanum* the type found in the mammal depends on the species.

tidae). These had been previously grouped in the order Protomonadina of Blochmann—a heterogeneous assemblage with only lack of plastids and few (1–4) flagella to unite them. The Bodonidae as defined by Hollande (1952) corresponded to the Heteromastigoda of Lankester's (1909) treatise and included several colorless biflagellates with heterodynamic flagella (e.g., *Cercomonas*) whose ultrastructure now sets them well apart from the kinetoplastids. Honigberg's order also embraced (as bodonines) the proteromonads now recognized as an independent order, Proteromonadida (q.v.).

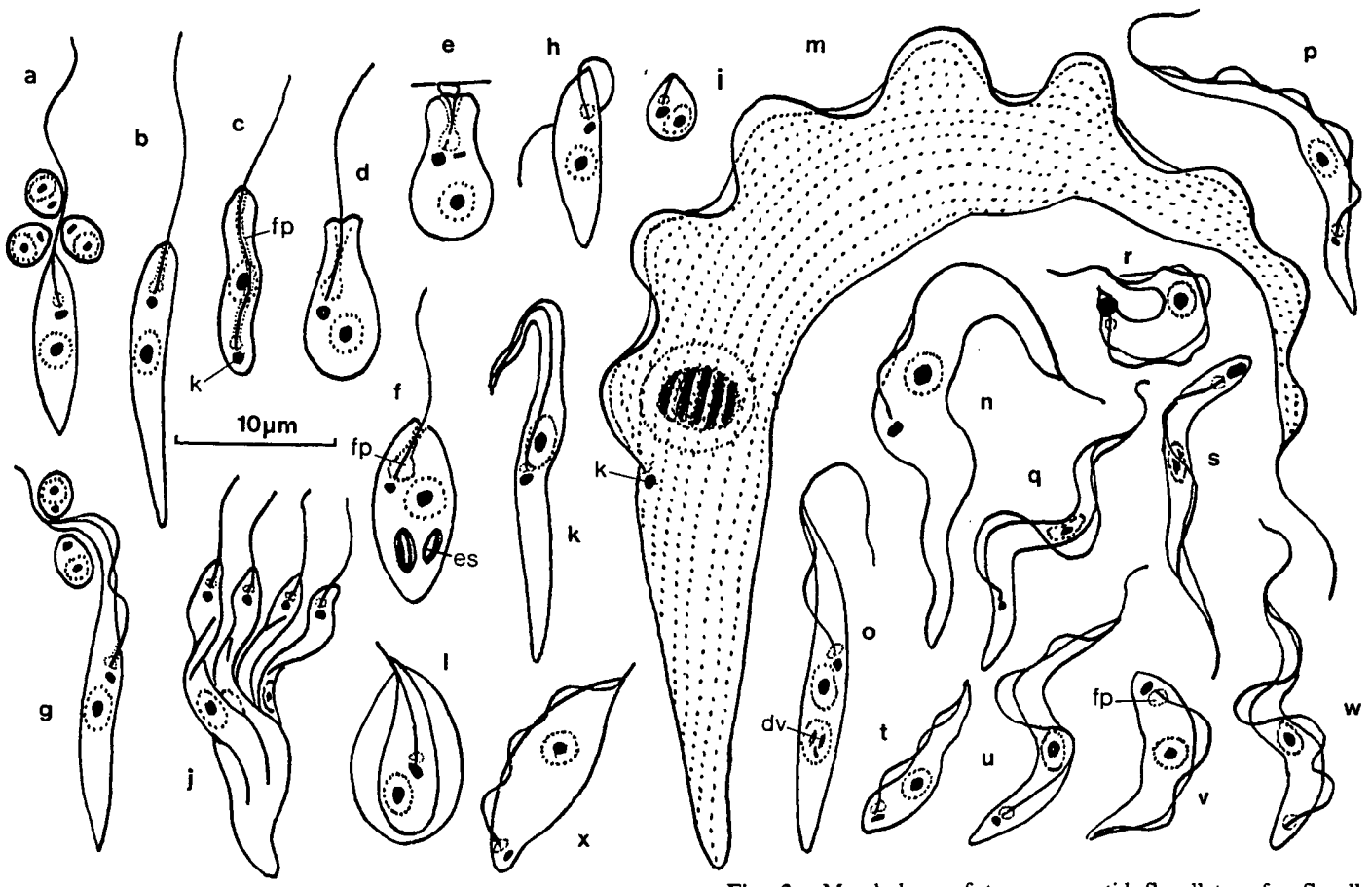

Fig. 2. Morphology of trypanosomatid flagellates: fp, flagellar pocket; PM, promastigote; AM, amastigote; OPM, opisthomastigote; CHM, choanomastigote; EPM, epimastigote; TPM, trypomastigote; other abbreviations as in Fig. 1. a, *Leptomonas oncopelti* (PM) with "straphanger" cysts; b, *Herpetomonas muscarum* (PM); c, *H. muscarum* (OPM); d, *Crithidia fasciculata* (CHM, nectomonad); e, *C. fasciculata* (CHM, haptomonad); f, *C. oncopelti* (CHM) with endosymbionts (es); g, *Blastocrithidia familiaris* (EPM) with cysts; h, *Leishmania major* (PM); i, *L. major* (AM); j, *Phytomonas elmassiani* (PM), multiple fission stage in plant latex; k, *Rhynchoidomonas drosophilae* (TPM); l, *Endotrypanum schaudinni* (EPM) in sloth red cell; m, *Trypanosoma grayi* (TPM) from crocodile blood; n, *T. (Megatrypanum) cyclops* (TPM) from blood of Macaque; o, *T. cyclops* (EPM, with pigment in digestive vacuole) from culture; p, *T. (Herpetosoma) musculi* (TPM) from mouse blood; q, *T. (Tejeraia) rangeli* (TPM) from human blood; r, *T. (Schizotrypanum) dionisii* (TPM) from pipistrelle bat; s, *T. (Duttonella) vivax* and t, *T. (Nannomonas) congolense* (TPM), both from cattle blood; u, *T. brucei* (TPM, slender bloodstream form); v, *T. brucei* (TPM, short stumpy form); w, *T. evansi* (TPM, dyskinetoplastic) from camel; x, *T. (Pycnomonas) suis* (TPM) from pig blood. (After Vickerman and Preston, 1976).

Kinetoplastids were recognized as pathogens when Evans in 1880 discovered the trypanosome (*Trypanosoma evansi*) that causes the disease surra of horses and camels in India. For a history of knowledge of kinetoplastids as agents of disease see Zuckerman and Lainson (1977) for leishmanias, Hoare (1972) for trypanosomes, and Dollet (1984) for *Phytomonas*.

Practical Importance

Several kinetoplastids are of medical, veterinary, or agricultural importance on account of their pathogenicity to man, his domestic animals, and even his crop plants. Three approaches have been tried in the control of diseases caused by the kinetoplastids: drugs, vaccination, and vector elimination. Insecticides and other vector control methods are currently in more widespread use since drugs often give unsatisfactory results and vaccination is usually not possible (see *The Surface Membrane and Antigenic Variation*, p. 228). Vector control has disadvantages, however in that these programs are expensive, effective spraying is nearly impossible, and often knowledge (e.g., location of vector breeding sites) is lacking. The threat of trypanosomiasis (nagana) to cattle in Africa south of the Sahara thwarts the utilization of forest grassland for human food production; native vegetation unsuitable for human consumption could be converted by ruminants into high quality protein, but cattle are prevented by the disease from thriving in these places. The trypanosomes responsible—*Trypanosoma brucei*, *T. congolense*, and *T. vivax*—are transmitted by tsetse flies (*Glossina* spp.) in whose internal organs the parasites undergo a complex cycle of development before they reach the infective metacyclic stage in the fly's saliva (cyclical transmission, Fig. 5, Table 4, and *Life Cycles*, p. 226). In tsetse-infected areas, cattle die from severe anemia and other pathogenic consequences of trypanosome infection.

Outside the tsetse belt of Africa, *Trypanosoma evansi* (a monomorphic descendant of *T. brucei*—see Tables 3 and 4 and

TABLE 3. Subgenera of *Trypanosoma* from mammals: Distinguishing features and representative species

Subgenus	*Mammalian Trypomastigote Distinguishing Features*	*Representative Species*	*Behavior in Mammal*
Section: Stercoraria			
Megatrypanum[a] Hoare 1964	Lrge TPM(40–100μm); long pointed PE; medium, nonterminal kinetoplast; long FF.	*T.(M.)theileri*	Division in BS as EPM; ND TPM.
		T.(M.)melophagium	Division in BS unknown
Herpetosoma[b] Doflein 1901	Medium TPM(21–36μm); long pointed PE; large rodlike nonterminal kinetoplast; long FF.	*T.(H.) lewisi*	Multiple fission in visceral capillaries as EPM; ND TPM
		T.(H.) musculi[f]	
Schizotrypanum[c] Chagas 1909	Small TPM(15–24μm); short pointed PE; large subterminal kinetoplast; long FF.	*T.(S.) cruzi*	Division as intracellular AM in muscle or MNP; ND TPM in blood
		T.(S.) dionisii[g]	Division as intracellular EPM in 'pseudocysts' derived from MNP
Incertae sedis:			
Tejeraia[d] Anez 1982	Medium TPM (25–35μm); long pointed PE; medium nonterminal kinetoplast; long FF.	*T.(T.)rangeli*[h]	ND TPM only, known in mammal
Section: Salivaria			
Duttonella[e] Chalmers 1908	Medium TPM(21–26μm); blunt, small (14–17μm) to rounded PE; large terminal kinetoplast; long FF.	*T.(D.) vivax*	Division in BS as medium TPM; wholly intravascular
		T.(D.) uniforme	As *T. vivax* but TPM smaller
Nannomonas[e] Hoare 1964	Small TPM(12–18μm); blunt PE; medium, subterminal marginal kinetoplast; no FF.	*T.(N.) congolense*	Division in BS as TPM attached to endothelia
		T.(N.) simiae	As *T. congolense*, but long and short forms occur (pleiomorphic)
Pycnomonas[e] Hoare 1964	Small TPM(8.5–19μm); very short pointed PE; small subterminal kinetoplast; short FF.	*T.(P.) suis*	Division in BS as TPM
Trypanozoon[e] Lühe 1906	Pleiomorphic TPM; long slender forms (mean 30μm) with long FF and short stumpy forms (mean 18μm) with no FF; small subterminal kinetoplast	*T. brucei*	Dividing slender TPM in blood, lymph, CT Nondividing stumpy TPM in blood and lymph
		T. evansi *T. equiperdum*	As *T. brucei* but monomorphic

Abbreviations: AM-amastigote; BS-bloodstream; CT-connective tissues; EPM-epimastigote; FF-free flagellum; ND-nondividing; MNP-mononuclear phagocyte; PE-posterior extremity; TPM-trypomastigote

References: [a]Wells 1976; [b]Molyneux 1976, Mansfield 1977; [c]Marinkelle 1976; Miles 1979; [d]D'Alessandro 1976; Anez 1982; [e]Hoare 1972.

Notes: [f]Parasite of house mouse, *Mus musculus*; [g]Parasite of bats (*Pipistrellus*). For hosts of other species, see Table 4. [h]Originally included by Hoare (1972) in subgenus *Herpetosoma*, removed to subgenus *Tejeraia* by Anez (1982) on account of anomalous life cycle.

TABLE 4. Trypanosomes of mammals: Hosts, transmission and relation to disease of man and domestic animals

Species	*Main Hosts/Reservoirs*	*Vectors/Transmission*	*Disease*	*Geographical Distribution*
T. (Megatrypanum) theileri	Cattle	Tabanid flies (C;PS)	Nonpathogenic	Cosmopolitan
T. (M) melophagium	Sheep	Sheep ked (C;PS)	Nonpathogenic	Temperate areas
T. (Herpetosoma) lewisi	Rats	Rat fleas (C;PS)	Nonpathogenic	Cosmopolitan
T. (Schizotrypanum) cruzi	Man, opossums, armadillos raccoons, cats, dogs, rodents	Triatomine bugs (C;PS)	Chagas' disease	C. & S. America
T. (Tejeraia) rangeli	Man, dogs, cats, opossums, monkeys	Triatomine bugs (C;AS;SG)	None(pathogenic in vector only)	S. America
T. (Duttonella) vivax	Ruminants, equines, bushpig	Tsetse flies (C;AS; P)	Nagana	W., C. & E. Africa
T. (D) vivax viennei	Cattle	Tabanid flies (M)	None or mild	S. America, W. Indies, Mauritius
T. (D) uniforme	Cattle, pigs, antelopes	Tsetse flies (C;AS;P)	None or mild	C. & E. Africa
T. (Nannomonas) congolense	Ruminants, equines, pigs, carnivores, rarely camels	Tsetse flies (C;PS;P)	Nagana	W., C. & E. Africa
T. (N) simiae	Pigs, camels, monkeys	Tsetse flies (C;AS;P)	Acute in pigs	W., C., E. & parts of S. Africa
T. (Pycnomonas) suis	Pigs, warthog	Tsetse flies (C;AS;SG)	Acute in piglets	C. & E. Africa
T. (Trypanozoon brucei brucei	Ruminants, camels, pigs, carnivores	Tsetse flies (C;AS;SG)	Nagana	W., C., E. & parts of S. Africa
T. (T) brucei rhodesiense	Man, cattle, antelopes; carnivores	Tsetse flies (C;AS; SG)	Acute sleeping sickness	E. Africa
T. (T) brucei gambiense	Man, domestic pig, dog	Tsetse flies (C;AS;SG)	Chronic sleeping sickness	W. Africa
T. (T) evansi	Camels, equines, ruminants, Indian elephant, carnivores, vampire bat	Tabanid flies (M); Vampire bats	Surra	N. Africa, S. Asia, E. Indies, Mauritius, C. & S. America
T. (T) evansi equinum	Equines, ruminants, pig, capybara, vampire bat	Tabanid flies (M); Vampire bats	Mal de caderas	C. & S. America
T. (T) equiperdum	Equines	Venereal contact	Dourine	N. Africa, S. Europe, S. America

Abbreviations: Transmission C–cyclical; M–mechanical. Metacyclics formed in: AS–anterior station, (mouth parts of vector, for injection with saliva); P–in proboscis; SG–salivary glands; PS–posterior station (hindgut of vector for deposit with feces).

Evolutionary History, p. 234—and *T. vivax* may be spread by biting flies (especially tabanids) without a cycle of parasite development in the vector. The principal victims of such mechanical transmission are the draft animals of the unmechanized farming world, horses and camels in particular (Table 4). Vampire bats (*Desmodus* spp.) may act as mechanical vectors of *T. evansi*, the trypanosomes moving from blood of ungulate to that of bat and vice-versa via the bat's saliva. The now rare equine disease dourine is due to *T. equiperdum*—a derivative of *T. evansi* that is transmitted venereally between horses. Carnivores (e.g., lions, leopards, dogs) can acquire infection with the salivarian trypanosomes by eating the carcasses of infected ungulates (Hoare, 1972).

African human trypanosomiasis or sleeping sickness is a serious and usually fatal disease caused by genetic variants of *T. brucei* that are given subspecific designations. *T.b. gambiense* causes the chronic West African disease and is transmitted cyclically by "wet" (riverine) tsetse e.g., *Glossina palpalis*. *T.b. rhodesiense* causes the acute East African form of the disease; its vectors are "dry" (savannah) flies e.g., *G. morsitans*. Trypanosome infections result in damage to vascular and immune systems, but whether damage is direct or indirect is controversial (Tizard, 1985). Sleeping sickness has about 200 natural foci scattered over 38 African countries; 50 million people are at risk from epidemic resurgence of the disease, which may be induced by increased man-fly contact, changes in human habits or habitat, or genetic change on the part of the trypanosome. Both forms of disease are zoonoses with reservoirs in domestic pig for the gambian disease and in cattle or antelopes (bushbuck, hartebeeste) for the rhodesian parasite.

Chagas' disease caused by *T. cruzi* affects about 10 to 12 million people from South and Central America, where it is the most important etiological agent of cardiac disease in those under 40 years of age. The trypanosome multiplies intracellularly in the heart and other muscles, emerging into the blood as a trypomastigote to invade other cells or for uptake by the blood-sucking triatomine bug vector for cyclical transmission. The disease is transmitted through the bug's feces, which are contaminated with metacyclic trypomastigotes and eliminated by the bug after feeding. The metacyclics penetrate the vertebrate host through skin lesions or normal eye mucous membranes. In rural areas these bugs readily invade dilapidated housing where they hide in crevices in wall and thatch, emerging at night to feed. Blood transfusion accounts for transmission in urban areas. The disease is a zoonosis with a reservoir in several mammals (Table 4). It has an acute phase (1–2 months) followed by a chronic phase that may last for years (Santos-Buch and Costa, 1985). An autoimmune reaction is suspected in pathogenesis of Chagas' disease, but this is controversial (Hudson and Britten, 1985).

Leishmaniases in man is caused by a complex of six species and 15 or 16 subspecies of *Leishmania* (WHO, 1984). All leishmanias are transmitted by sand flies (Phlebotominae), the ingested amastigotes transforming to promastigotes in the insect gut (Table 5); infection is usually by fly bite. As the morphology of these parasites in their macrophage host cell is similar, species recognition depends on the clinical features of the disease caused and on isoenzyme electrophoresis and DNA direct analysis techniques in the laboratory (see *Classification Schemes*, p. 232). Clinical manifestations of leishmaniasis in-

TABLE 5. Species of *Leishmania* causing disease in man

Species	*Disease/Lesion*	*Distribution*	*Reservoir Hosts*
OLD WORLD SPECIES[a]			
L. tropica[c]	Dry cutaneous; chronic; urban OS; (LR)	Europe, Asia, N. Africa	Dogs
L. major	Wet cutaneous; acute; rural OS.	Asia, Africa	Rodents
L. aethiopica	Dry cutaneous OS; (mucocutaneous, DCL)	Ethiopia, Kenya	Hyrax
L. donovani donovani[d]	Visceral Kala azar (PKADL)	Africa, Asia	None known
L. donovani archibaldi[d]	Visceral	Kenya, Sudan	Rodents
L. donovani infantum[c]	Infantile visceral	Mediterranean	Dogs, foxes porcupine
NEW WORLD SPECIES[b]			
L. mexicana mexicana	Cutaneous (Chiclero's ulcer)	Central America	Rodents
L. mexicana amazonensis[c]	Cutaneous (DCL)	Brazil	Rodents, oppossums
L. mexicana pifanoi[c]	Cutaneous (DCL)	Venezuela, Brazil	Rodents
L. braziliensis braziliensis	Mucocutaneous; (Espundia) metastasizing	S. America (exc. Argentina, Chile)	Rodents
L. braziliensis guyanensis	Cutaneous (Pian bois); metastasizing	Guyanas, N. Brazil	Edentates
L. braziliensis panamensis	Cutaneous; metastasizing	Panama	Forest rodents, edentates, procyonids
L. peruviana[e]	Cutaneous (Uta)	Peruvian Andes	Dogs
L. chagasi[c]	Infantile visceral	S. America	Foxes

[a] Vectors, species of *Phlebotomus*
[b] Vectors, species of *Lutzomyia*
[c] Incidentally parasites of man
[d] Primarily parasites of man (anthroponotic)
[e] Only New World species not associated with forest habitat.

OS–Oriental Sore; (DCL)–disseminative cutaneous leishmaniasis in some individuals in absence of cell-mediated immunity; (LR)–Leishmaniasis recidivans, chronic, nonhealing lesion not responding to treatment; (PKADL)–post-Kala azar dermal leishmaniasis, skin lesions occurring after apparent cure of visceral leishmaniasis. Abbreviations in brackets represent occasional complications.

clude both cutaneous and visceral forms of the disease as summarized in Table 5. The most serious disease, Kala azar (visceral), involves the macrophages of liver, spleen and bone marrow, causing a fatal anemia that, left untreated, has a high mortality rate. The danger these diseases represent for the health of children should be especially emphasized, as infants are more vulnerable and risk of failure of diagnosis is high.

Leishmanial infections in man induce both humoral and cellular responses, but the balance of their expression varies with the type of disease (Mauel and Behin, 1982).

Other kinetoplastids of practical importance are the fish-parasitizing bodonids (Becker, 1977) and species of *Phytomonas*, parasitizing palm trees and coffee plants (Dollet, 1984) (see Tables 1 and 2 and Habitats and Ecology, below). In recent years, the kinetoplastids have assumed importance in molecular biology by virtue of the unusual nature and enigmatic function of kinetoplast DNA (kDNA, see *The Kinetoplast*, p. 226), and as a result of detailed studies on the genetic basis of antigenic variation—the process whereby trypanosomes evade their host's immune response. These studies are among the most sophisticated on eukaryotic gene expression being conducted today.

HABITATS AND ECOLOGY

Completely free-living kinetoplastids are all phagotrophic bodonids (Table 1), and many are common in infusions. Most feed on bacteria while creeping along surfaces, using the anterior flagellum for locomotion and for propelling food particles toward the cytostome while the posterior flagellum functions as a "skid" that permits contact of the kinetoplastid with the substratum. These organisms are most abundant in organic-rich environments, but many species have strict oxygen requirements and tend to accumulate at a characteristic distance from the air/water interface. Common as coprozoic organisms, they have often been mistaken for parasites in stools or urine samples from a variety of patients (Vickerman, 1978a). Since bodonids do not survive body temperature, these instances must result from contamination of the samples with free-living organisms. Most free-living bodonids can form thin-walled cysts (Fig. 1d)

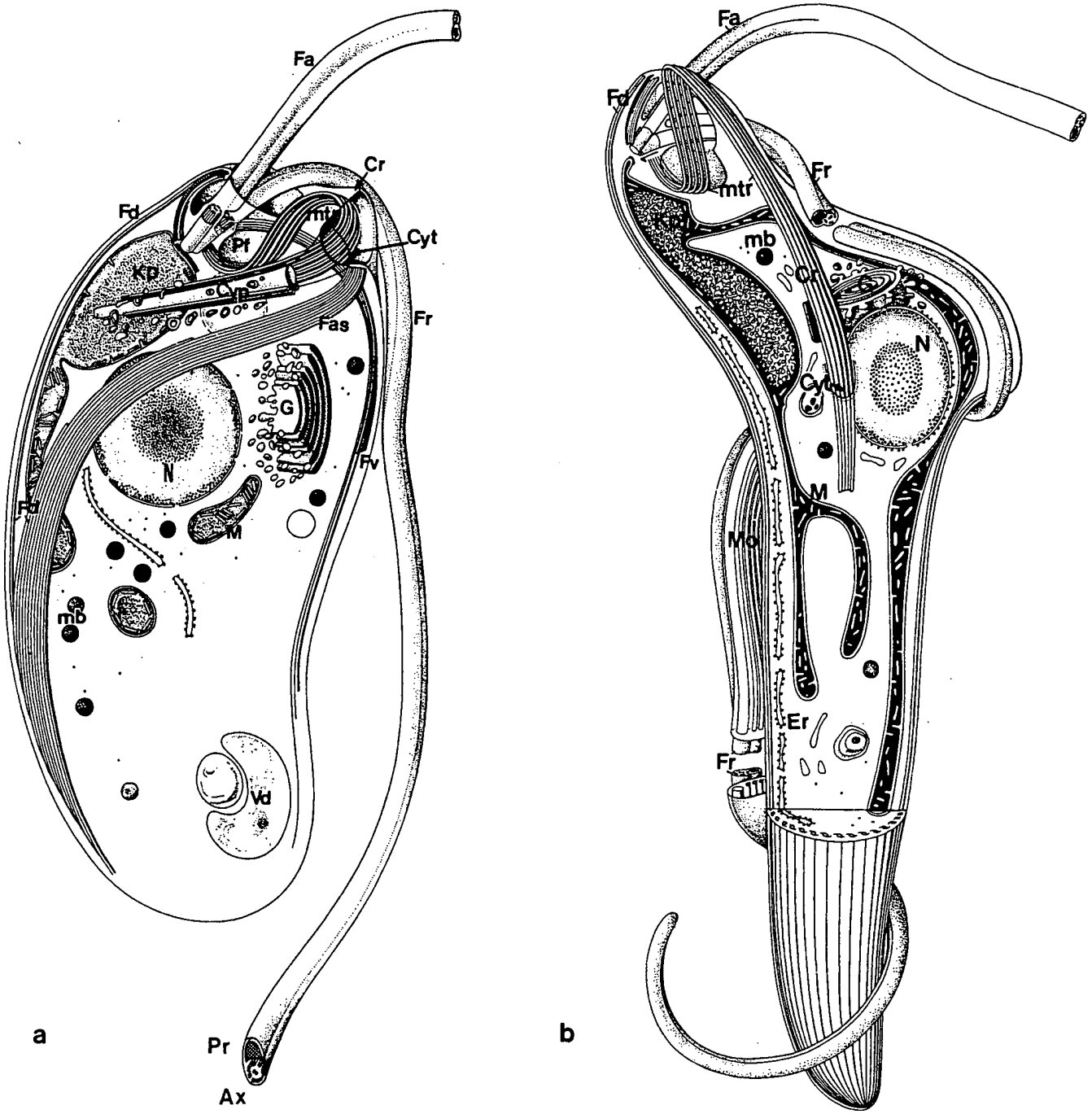

Fig. 3. Ultrastructure of the bodonid flagellates.

a. Schematic dissection of trophozoite of *Bodo caudatus* seen in right lateral view. The two flagella (Fa, anterior flagellum; Fr, posterior (recurrent) flagellum) arise from a flagellar pocket (Pf); each has a paraxial rod (Pr) in addition to an axoneme (Ax) in its shaft. Their kinetosomes are separated from the kinetoplast (Kp) region of the mitochondrion by a dense plate; the single mitochondrion (seen as several profiles (M), forms a figure eight in the cell. The cytoskeleton consists of 3 microtubular bands: Fd, the dorsal fiber arising from the dorsal Pf wall and connected to the kinetosome of Fa by 3 microtubules; Fv, the ventral fiber, connected to the kinetosome of Fr and passing along the left side of the Pf wall; mtr, a band of microtubules from the same kinetosome reinforcing the preoral crest (Cr) and deflected inwards at the cytostome (Cyt) to support the cytopharynx (Cyp). A band of microtubules Fas, associated with the cytostome, passes along the right side to become incorporated into a sheet of microtubules along with the dorsal fiber. The contractile vacuole (which empties into Pf) is seen behind Cyp and Fas. N, nucleus; Er, endoplasmic reticulum; G, golgi apparatus; Vd, digestive vacuole; mb, microbody (probably a glycosome). (From Brugerolle *et al.*, 1979).

b. Schematic dissection of *Cryptobia* (*Trypanoplasma*) *borreli* from blood of gold fish (*Carassius auratus*). Although similar in structure to *Bodo*, the posterior (recurrent) flagellum (Fr) adheres to the body and in beating draws up its surface into an "undulating membrane" (Mo). The body is enclosed in an incomplete corset of microtubules corresponding to the Fd and Fv of *Bodo*. The preoral crest (Cr) is extremely long and supported by a microtubule band (Mtr) which plunges in the cytostome/cytopharynx about one third of the way along the body. Label abbreviations as in a. (From Brugerolle *et al.*, 1979).

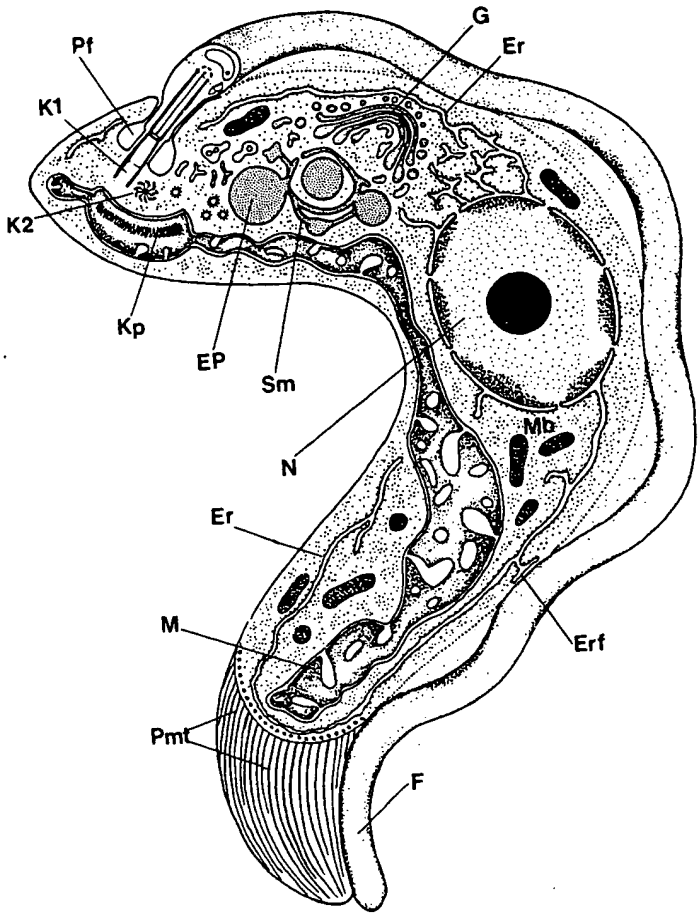

Fig. 4. Ultrastructure of a trypanosomatid flagellate, *Trypanosoma congolense*, from the blood of its mammalian host. The single flagellum (F) arises from a flagellar pocket (Pf) and corresponds to the anterior flagellum of bodonids; in *Trypanosoma* it adheres to the body to form an undulating membrane. Close to its kinetosome (K1) lies a barren kinetosome (K2), which is all that remains of the recurrent flagellum. The kinetoplast (Kp) lies in an expansion of the single mitochondrion (M), which has tubular cristae. Pellicular microtubules (Pmt) form a corset encasing the entire body. Granular endoplasmic reticulum (Er) is abundant in the cytoplasm as are glycosomes (microbodies, Mb); a specialized cisterna of ER (Erf) runs along the entire length of the undulating membrane. Between the nucleus (N) and flagellar pocket the cytoplasm contains a smooth-membraned reticulum (Sm) with saclike dilations; this probably forms a sequestering and digestive system for protein (EP) endocytosed from the flagellar pocket. G, golgi apparatus. (After Vickerman, 1969).

and so can pass through the gut of vertebrates to hatch out in the voided feces. One of the most ubiquitous bodonids, *Bodo saltans*, however, appears to be unable to form a cyst.

Epizoic bodonids include *Cephalothamnium cyclopum* (Fig. 1h), which forms colonies attached to its copepod host. Many bodonids live on the gills and skin of fish where they adopt a variety of life-styles. *Cryptobia branchialis* and *C.* (=*Bodomonas*) *concava*, which attach by their recurrent flagella, are claimed to feed on bacteria and dead cells in the gill mucus and so should be transferred to the genus *Procryptobia*. *Ichthyobodo necator* (=*Costia necatrix*), an important parasite of freshwater fish and especially of alevins in fish hatcheries (Becker, 1977), attaches to epithelial cells by an anterior rostrum and ingests cell cytoplasm (Fig. 1f). A dispersive free-living phase (Fig. 1g) lacks the rostrum and probably does not feed.

Several species of *Cryptobia* have been described from the foregut of marine teleosts, and the esophagus or stomach may be thickly carpeted with these bodonids (Becker, 1977). Gut cryptobias are common, too, in freshwater planarians (e.g., *C. dendrocoeli* in *Dendrocoelum lacteum*). *C. iubilans* of the gut of cichlid fishes (Nohýnkovà, 1984) can spread to other organs (gall bladder, spleen, ovary, liver) where it appears to multiply inside macrophages—within a parasitophorous vacuole (cf. *Leishmania* spp., *vide infra*). Transmission of these gut cryptobias is probably via a free-swimming aquatic phase, but the cryptobias reported from the gut of terrestrial gecko lizards (Bovee and Telford, 1962) may have an encysted stage that ensures transmission. Copulation seems the most likely means of transmission of the cryptobias (e.g., *C. vaginalis:* Fig 1i,j) found in the female reproductive tract of invertebrates. *Cryptobia helicis* of terrestrial and aquatic gastropods attaches to the microvillar surface of its host organ by tentaclelike outgrowths of its anterior flagellum (Current, 1980). All true cryptobias feed by pinocytosis through a cytostome-cytopharynx.

Most of the monogenetic trypanosomatids of arthropods (Wallace, 1979) are strictly localized in the guts of their larval or adult hosts. *Crithidia* species tend to be found in the hindgut, where they attach to the chitinized lining by their shortened flagella (Fig. 2e). Such attached or "haptomonad" stages (Molyneux, 1983) are also found in *Leptomonas*, *Herpetomonas*, and *Blastocrithidia*. More often species of these genera are found as free-swimming "nectomonads" in the gut lumen, either within or outside the peritrophic membrane. Occasionally the Malpighian tubules are invaded (e.g., *Rhynchoidomonas* spp.), or the hemocoel and salivary glands. The developmental stages of digenetic trypanosomatids occupy similar sites. Those trypanosomes (Stercoraria, see Table 3) that produce the infective metacyclic stage in the hindgut have no reason to leave the alimentary tract. Although *Phytomonas* spp. (Table 2) and *Trypanosoma rangeli* (Table 4) invade the hemipteran's salivary glands from the gut via the hemocoel, the evidence that *Trypanosoma brucei* follows the same route in *Glossina* is poor. Migration to the glands most likely is via the proboscis (Vickerman, 1985). Attached stages in the trypanosomatid life cycle may densely carpet the body surface to which they attach. Although the environment of these organisms is aerobic, anaerobic conditions may exist locally—especially in the parasite-packed insect hindgut.

Transmission of the monogenetic trypanosomatids is apparently through the nectomonad form in the aqueous environment, and more rarely via an encysted stage (see *Life Cycles*, p. 226) contaminating food. These trypanosomatids survive the diapause in some endopterygote insects but their location in the pupa is uncertain. Transovarian transmission to offspring of *Leptomonas jaculum* and *Blastocrithidia euryophthalmi* has been reported (McGhee and Cosgrove, 1980). Predatory Hemiptera may "adopt" the trypanosomatids of their prey

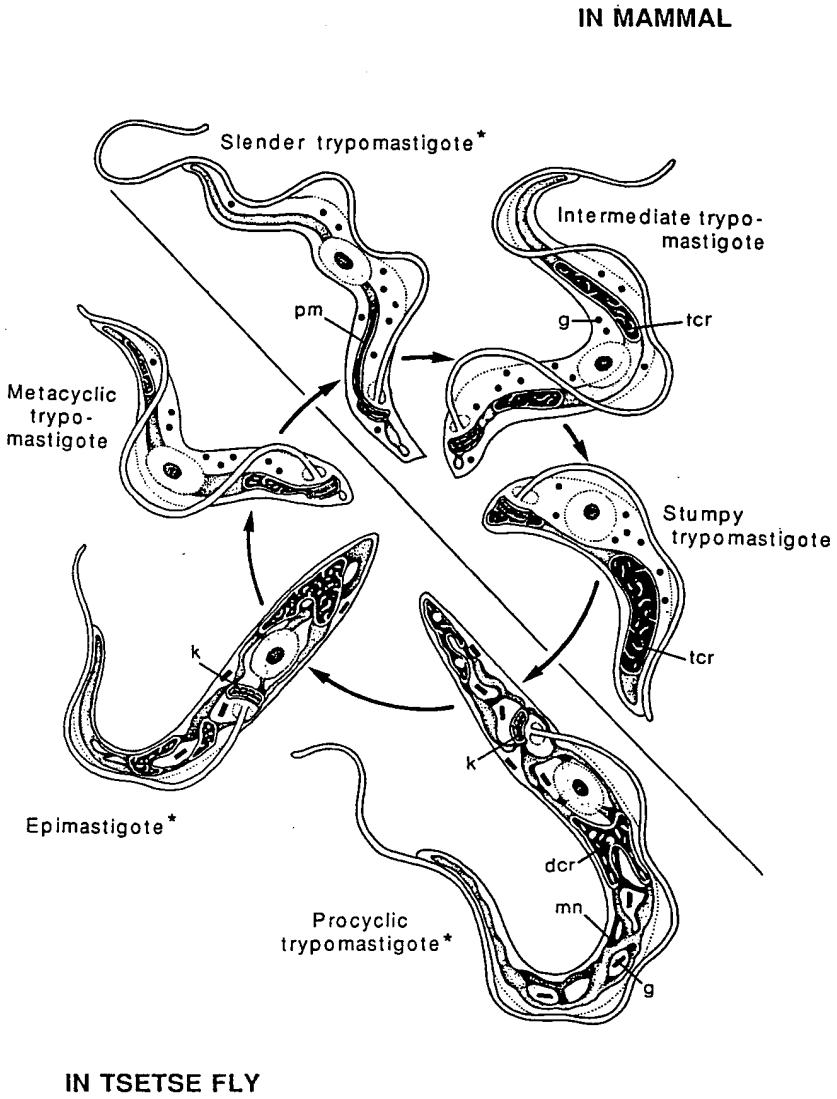

Fig. 5. Life cycle of *Trypanosoma brucei* showing changes in position of kinetoplast, and in the form of the mitochondrion and glycosomes (g). The mitochondrion is shown cut open to display its interior. Stages in which division occurs are marked with an asterisk.

In mammalian host: Long slender trypomastigotes divide in blood and tissue fluids giving rise to nondividing intermediate and short stumpy trypomastigotes. Slender forms have a simple promitochondrion (pm) with few or no internal cristae; a functional respiratory chain is missing and the parasite depends entirely on glycolysis for energy production. Glycolytically-reduced NADH is reoxidized by a dihydroxyacetone phosphate/glycerol 3-phosphate oxidase shuttle in combination with a cyanide- and azide-insensitive glycerophosphate oxidase, located in the mitochondrial membrane, which reacts with O_2 without the intervention of cytochromes and without coupling to ATP synthesis. Transformation to the stumpy form is accompanied by acquisition of proline and α-ketoglutarate oxidase activities as the mitochondrion swells and develops tubular cristae (tcr).

In the insect host: On entering the tsetse midgut the mitochondrion expands into a network (mn) as the post-kinetoplast and prenuclear regions of the trypomastigote increase in size. Succinoxidase activity appears and proline and α-ketoglutarate oxidases are augmented as the trypanosome switches from a glucose-based energy metabolism to one based on proline. Later, in the midgut, discoid cristae (dcr) replace the tubular type and a complete cytochrome chain appears; the chain may be branched at cytochrome *b* allowing flow of electrons to oxygen either via cytochromes *a*-a_3 (cytochrome oxidase). On migration from the fly midgut to the salivary glands, the trypanosome becomes a multiplicative epimastigote with prenuclear kinetoplast (k) and tubular mitochondrial cristae. On transformation to the metacyclic trypomastigote the mitochondrion becomes a linear structure again; as yet nothing is known about the respiration of the salivary gland stages in the life cycle.

Note change in form of glycosomes (g) from spherical to bacilliform structures and *vice versa* (based on Vickerman, 1971, 1985; Opperdoes, 1985).

insects, and the so-called "*Leishmania* spp." found as promastigotes in the hindgut and rectum of chameleons and other lizards may be similarly acquired.

Phytomonas spp. inhabit the latex or phloem vessels of their plant hosts. The details of transmission of the pathogenic species in the phloem of coffee and oil of coconut palms are unknown; the nonpathogenic species are transmitted cyclically by phytophagous heteropterans (Lygaeidae: Dollet, 1984).

The digenetic trypanosomes and trypanoplasms (*Cryptobia* spp.) of the blood of aquatic poikilotherms are transmitted by leeches. After ingestion in the bloodmeal of the annelid, the parasites undergo a cycle of morphological changes culminating in the production of metacyclic flagellates in the proboscis sheath (Lom, 1979), ready to infect the vertebrate host.

The trypanosomes are best known as free-swimming flagellates in vertebrate blood. However, they may become sequestered in the capillaries of certain organs, especially during the multiplicative phase (e.g., *T. lewisi*), attach to peripheral capillary endothelia (e.g., *T. congolense*), or leave the vascular system and invade the lymphatics and connective tissue fluid (e.g., *T. brucei, T. evansi*; see Table 3 and Losos and Chouinard, 1979). *T. cruzi* multiplies as amastigotes inside host muscle cells or mononuclear phagocytes. Like *Leishmania* spp. in phagocytes, it must avoid killing and digestion by the host macrophage's lysosomal enzymes (Thorne and Blackwell, 1983). *T. cruzi* surmounts this problem by escaping from the parasitophorous vacuole in which it was engulfed into the cytoplasmic matrix. The leishmanias somehow modify the environment so that although lysosomes fuse with the vacuole their contents are inactivated by the parasite. *Endotrypanum* species, which like leishmanias are also transmitted by phlebotomine sandflies, avoid the lysosome threat in the mammal by inhabiting erythrocytes (Table 2).

CHARACTERIZATION AND RECOGNITION

Definition of Order Kinetoplastida

Flagellates with one or two flagella, each typically possessing a paraxial rod in addition to the axoneme and arising from a flagellar pocket or pit. They contain a single mitochondrion,

typically extending the length of the body—linear, branched, or reticulate—that contains a prominent DNA kinetoplast usually located close to the flagellar kinetosomes that insert on (or close to) the mitochondrial outer membrane. Mitochondrial cristae are discoid or tubular. Primary enzymes of the glycolytic pathway are housed in glycosomes—microbodylike organelles unique to the order. The cytoskeleton is composed of supporting pellicular microtubules (microtubular arrays associated with the plasma membrane). A microtubule-surrounded cytopharynx is present in many species, presumably secondarily lost by many osmotrophic forms. Pseudopodia are absent. There is a single vesicular nucleus with a prominent nucleolus. Nuclear division with intranuclear spindle, lacking polar structures. Condensed chromosomes are not visible. No plastids or storage carbohydrate bodies are present, but lipid globules commonly are present. The golgi apparatus is typically in the region of the flagellar pocket, but is not connected to kinetosomes. Contractile vacuoles, if present, empty into the flagellar pocket. Reproduction typically is by binary fission; meiosis, gametes, and syngamy are undemonstrated but have been inferred in some species. Kinetoplastids are free-living and parasitic. Encystation is common among free-living forms, rare in parasitic forms.

The ultrastructure of representative bodonids and trypanosomatids is depicted in Figures 3 and 4.

Life Cycles

The parasitic kinetoplastids often have elaborate life cycles; the most complex are those in which the parasites alternate between two hosts and undergo morphological changes as they progress from one environment to the next. These different developmental stages may be characterized by changes in kinetoplast form and position in the body.

Free-living bodonids alternate between a dividing trophozoite and an encysted stage in which the flagella may be partially resorbed, e.g., *Bodo caudatus*. However, in some the kinetoplast may become fragmented to give a polykinetoplastic cyst, e.g., *Procryptobia glutinosa* (Fig. 1c,d; Vickerman, 1978a), whereas other species lack an encysted stage altogether. Parasitic bodonids may show alternation of pankinetoplastic and eukinetoplastic stages in one host, e.g., *Cryptobia vaginalis* (Fig. 1i,j; Vickerman, 1977).

The simplest trypanosomatids exist as a single morphological stage that is parasitic, dispersive, and infective (e.g., *Leptomonas karyophilus*, parasitizing the ciliate macronucleus: Gillies and Hanson, 1963), while others (especially gut parasites of terrestrial insects) form aflagellate cysts, often by unequal division of the flagellated stage, and the cryptobiotic cysts serve in transmission. These cysts may show no cyst wall and their nuclei exhibit remarkable whorled chromatic configurations, suggesting an unusual cryptobiotic condition unknown in other protists (see Tieszen *et al.*, 1985).

Most trypanosomatids and parasitic bodonids lack an encysted stage but exhibit more than one morphological stage in the life cycle. Alternatively, the different stages in the life cycle may differ primarily in size, e.g., *Phytomonas* species retain the promastigote form: those of the midgut stage may be several times the size of those in salivary glands or in plant latex. In the monoxenous trypanosomatids the developmental cycle appears to be governed by the environment. Changes from short, broad, attached "haptomonads" (Fig. 2e) to long, actively swimming nectomonads (Fig. 2d) occur rapidly and reversibly. Transformation of promastigote to opisthomatigote in *Herpetomonas* species (Fig. 2b,c) has been induced by changes in pH and temperature as well as by inhibition of division or glucose transport (Wallace *et al.*, 1983); how such form changes relate to functional adaptation is unknown.

In the best studied life cycle, that of *Trypanosoma brucei*, the parasites' developmental cycle has been shown to be linked to a cycle of mitochondrial repression and activation (Vickerman, 1971). Repression occurs in the multiplicative slender trypomastigotes of the mammalian host, activation commences as multiplication ceases in the stumpy forms, reaching its peak in the tsetse fly midgut and declining after migration to the salivary glands so that the terminal metacyclic trypomastigotes are preadapted to invasion of the mammal once more (Fig. 5). Mechanically transmitted *T. brucei* lose the ability to activate the mitochondrion. The mitochondrial cycle, which is related to switches in substrate utilization, requires an intact kinetoplast (see below). Details of the cycle are given in the legend to Figure 5. The alternation of discoid and tubular mitochondrial cristae in this cycle argues against the assertion that cristal morphology is of phylogenetic significance.

Mitochondrial activation and repression are not the only feature of the cycle, however; changes occur in the glycosomes (see *The Glycosome*. p. 228) which house the enzymes of glycolysis, in the surface membrane of the trypanosome (see *The Surface Membrane*, p. 228) in relation to antigenic variation, and in infectivity. At least some of these changes are not directly reversible and so programming of the developmental cycle is strongly indicated (Vickerman, 1985).

The Kinetoplast

The kinetoplast stains with basic dyes, the Feulgen reaction, and other DNA-specific stains. The kinetoplast divides before the nucleus although the DNA replication periods (S phase) of the two organelles are synchronous. In transmission electron micrographs of sections, kDNA is seen as a disc, sphere, or rod composed of 2.5–3.0 nm thick fibrils. In trypanosomatids these are oriented anisotropically and are orthogonal to the face of the disc (Figs. 5,6,8). In bodonids the kDNA may be arranged in bundles of anisotropic fibrils as several distinct kinetoplasts (Fig. 7), or as bundles isotropically distributed in a less discrete region of the mitochondrial matrix (Vickerman and Preston, 1976).

The kDNA of trypanosomatids (reviewed, Englund, 1981; Stuart, 1983) is the only DNA known which is in the form of a network consisting of thousands of interlocked circles. The network structure causes the DNA to band rapidly in CsCl density gradients when deproteinized cell lysates are centrifuged. Spread networks are seen by EM to consist usually of two classes of circle, 25–50 maxicircles each 20–38 kb

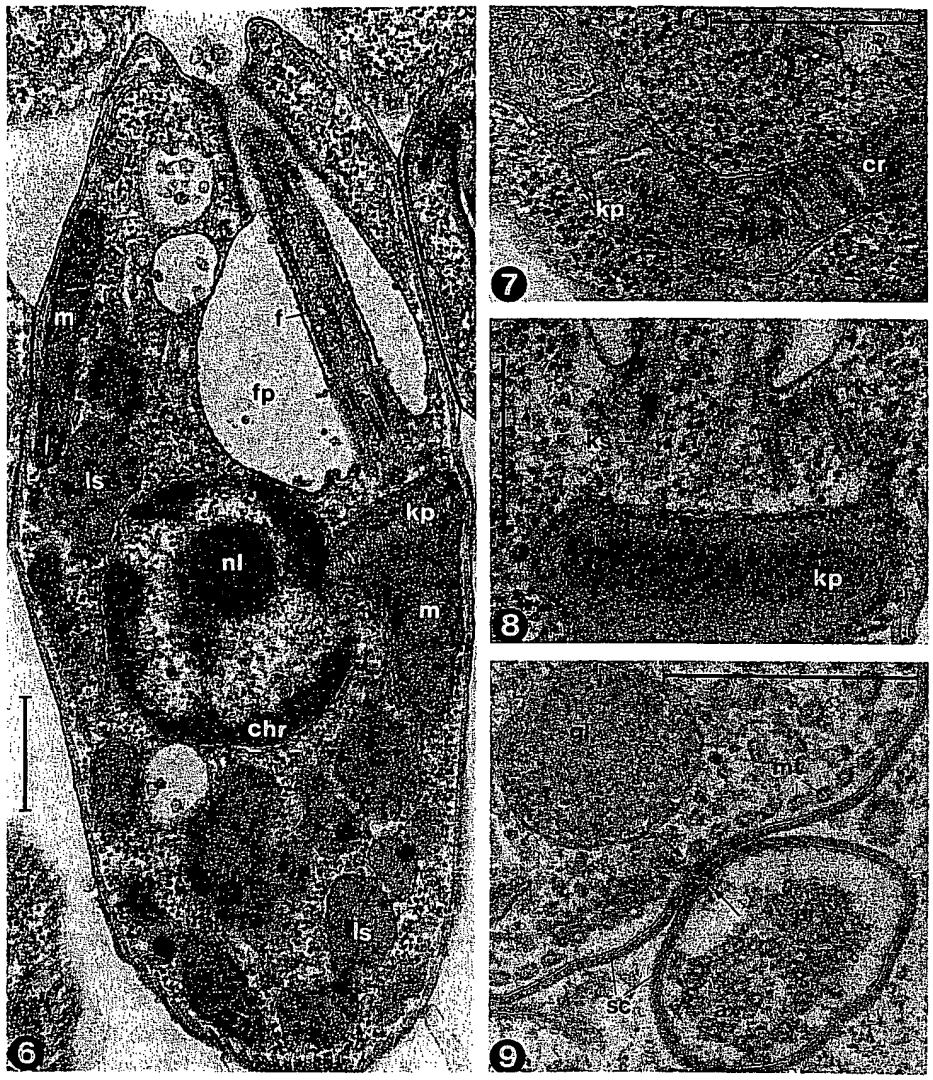

Fig. 6–9. Transmission electron micrographs. Scale on each = 0.5 μm. 6. Longitudinal section of *Leishmania mexicana* amastigote. Note: immotile flagellum (f) in flagellar pocket (fp); kinetoplast (kp) and profiles of mitochondrion (m); nucleus with nucleolus (nl) and chromatin (chr); numerous lysosomes ("megasomes") with heterogeneous contents (ls), believed to play a role in pathogenicity in this species. 7. Part of mitochondrion (m) of *Dimastigella trypaniformis* showing several bundles of kDNA fibrils (kp) interspersed with discoid cristae (cr). 8. Kinetoplast (kp) of *Herpetomonas muscarum* showing anisotropic arrangement of DNA fibrils and juxtaposition of parent and offspring kinetosomes (ks, ks′) to the mitochondrial envelope paralleling the disc-shaped kinetoplast. 9. Part of transverse section of metacyclic *Trypanosoma brucei* in region of flagellum. Note: axoneme (ax) with unusual septum on doublet microtubule B and paraxial rod (pr) in flagellum; surface coat of variable antigen (sc) on body and flagellum; pellicular microtubules (mt) nearby glycosome (gl); desmosome (arrowed) at discontinuity in microtubule layer.

(depending on species) and 5,000–10,000 minicircles each 0.46–2.5 kb. Maxicircles, which are similar to the mitochondrial DNAs of other eukaryotes, contain genes essential for mitochondrial biogenesis, and code for mitochondrial ribosomal RNA and subunits of some proteins involved in electron transport and ATP synthesis. They can be removed from the network by appropriate restriction enzyme digestion without affecting network structure. Minicircles are usually heterogeneous in nucleotide sequence and they evolve rapidly. In *T. evansi* and *T. equiperdum*, however, the minicircles are homogeneous, suggesting that an active mitochondrial system (absent from these two species) is somehow involved in generating and maintaining minicircle heterogeneity. They do not appear to be transcribed and probably do not have a conventional genetic function. Their most unusual feature is a region of "bent" DNA helix; some property of their nucleotide sequence causes the helix to assume a natural curvature leading to anomalous migration of restriction fragments on polyacrylamide gels (Marini *et al.*, 1982). Preliminary studies on the kDNA of *Bodo caudatus* suggests that the bodonines lack a kDNA network and that the kinetoplast is composed of a single class of DNA molecules (Hajduk *et al.*, 1986). Why trypanosomatids alone among eukaryotes should have their mitochondrial DNA organized in a network is not known, neither is the function of the minicircles—other than to hold the maxicircles together.

Replication of the kinetoplast raises problems not found with any other DNAs, owing to the network structure. Minicircles are released from the kDNA network for replication by a semiconservative mechanism (Hajduk and Englund, 1984); they then reattach to the periphery of the network. Release of minicircles occurs from the periphery inwards so that as replication proceeds the central zone of the network shrinks and the peripheral zone of reattached nicked minicircles enlarges as the network grows in size. In the G phase of the cell cycle the double-sized network divides into two. Maxicircles replicate by a different mechanism, though like minicircles each appears to replicate only once per generation. Single stranded "tails" (leading strands), emerging from maxicircle loops on the network periphery, appear to replicate and to become double stranded. The still-attached lagging strand appears to replicate *in situ*. Leading strand synthesis probably proceeds until the entire maxicircle genome is replicated whereupon the double

stranded branch is cleaved from the network to form a linear, free molecule that recircularizes and reattaches to the network (Hajduk and Englund, 1984).

The kinetoplast replicates during the division cycle after kinetosome reproduction is complete. The mitochondrial genome may be associated with the morphogenetic center of the kinetosome in order to ensure segregation of the maxicircles at division; the maxicircle gene products may be necessary for activation of the mitochondrion. Some mutants of *T. brucei* that cannot transform to the procyclic stage (i.e., undergo development in the insect) have maxicircle deletions or even complete absence of maxicircles. Some such mutants—the so-called dyskinetoplastic trypanosome lines—arise through disruption of network structure and dispersion of kDNA throughout the mitochondrion. They lack a stainable kinetoplast; typical maxicircles and minicircles cannot be isolated from them (Cuthbertson, 1981). Dyskinetoplasty can be induced by certain trypanocidal drugs (acridines, phenanthridines, diamidines) (Hajduk, 1978).

Dyskinetoplastic lines of *T. brucei* and its evolutionary derivatives when mechanically transmitted (see Evolutionary History, p. 234), can live indefinitely in the mammalian host—presumably because the mitochondrion is repressed in that phase of the life cycle and maxicircle gene products are not required. No other kinetoplastids can survive the dyskinetoplasty mutation (Hajduk, 1978). But dyskinetoplasty induced in pleiomorphic *T. brucei* does not necessarily halt the production of stumpy forms (Stuart, 1971). *T. cruzi,* moreover, which does not appear to have a phase of mitochondrial repression, if rendered dyskinetoplastic in its epimastigote stage, can differentiate into metacyclics that will invade mammalian cells and then proliferate as amastigotes (Deane and Kloetzel, 1969), suggesting that regulation of the mitochondrial respiratory system is at the post-transcriptional level.

The *T. cruzi* kinetoplast undergoes a marked change in size on transformation to the trypomastigote stage, but there is no evidence for amplification of DNA during the process (Newton, 1976a). There is some dispute over whether *T. brucei* kDNA is amplified during transformation of bloodstream trypomastigotes to procyclics (Newton, 1976a; Borst *et al.*, 1982), but in heteroxenous trypanosomatids a role for the kinetoplast during initial development in the insect host seems most likely.

The Glycosome and Other Biochemical Peculiarities

All kinetoplastids contain glycosomes—microbody (peroxisome)-like organelles—in their cytoplasm, diameter 0.2–1.0 μm, with a finely granular matrix and a bounding membrane similar in thickness to that of the endoplasmic reticulum (Fig. 3). In some cases crystalline bodies are present in the matrix. In the trypanosomatids these organelles have been shown to have a key role in energy metabolism in that they harbor the primary enzymes of the glycolytic pathway as well as two enzymes involved in the metabolism of glycerol-glycerol kinase and NAD^+-linked glycerol-3-phosphate dehydrogenase. Together, these enzymes account for the conversion of glucose and glycerol to 3-phosphoglycerate (Opperdoes, 1985). A major peculiarity of *T. brucei* and related species is that reoxidation of NADH generated in glycolysis is via a dihydroxyacetone phosphate/glycerol-3-phosphate shunt operating in conjunction with a terminal glycerophosphate oxidase. The oxidase is cyanide/azide insensitive and is located in the mitochondrial membrane. It reacts with oxygen directly and lacks coupling to ATP synthesis. The high activity of this glycerophosphate oxidase accounts for the high oxygen demands of bloodstream trypanosomes; the enzyme is inhibited by aromatic hydroxamic acids. A similar oxidase is found in some plants and fungi (Grant and Sargent, 1960; Opperdoes *et al.*, 1977).

Glycosomes also contain adenylate kinase, two enzymes of *de novo* pyrimidine synthesis, and, in insect stages of the life cycle, malate dehydrogenase and phosphoenolpyruvate carboxykinase, which participate in the glycolytic reoxidation of NADH and CO_2 fixation respectively. Glycosomes also have an important role in the synthesis of ether lipids as do mammalian peroxisomes (Opperdoes, 1985).

Glycosomes undergo a morphological change in the life cycle of *T. brucei*; the spherical structures of the metacyclic and bloodstream forms become bacilliform and more electron dense in the uncoated vector stages (Fig. 5), paralleling the repression of glycolysis in the insect host (Vickerman, 1985). The glycosome, being unique to Trypanosomatidae, may be an appropriate target for rational chemotherapy of diseases caused by these organisms. Although morphologically similar organelles occur in bodonids, no studies on their function are available.

Other biochemical peculiarities of Trypanosomatidae include a mini-exon sequence present in messenger RNAs (see *Nucleus and Transcription,* p. 231); naturally occurring chloramphenicol-resistant mitochondrial protein synthesis associated with a deletion in the mitochondrial rRNA gene (trypanosomatid mitochondrial rRNAs are 12S and 9S—the shortest rRNAs known); a different mechanism of metabolic regulation by cyclic AMP; a novel glutathione reductase of importance in protecting trypanosomes against toxic peroxides and other products of oxygen metabolism in the absence of catalase (see Opperdoes, 1985 for refs.).

The Surface Membrane and Antigenic Variation

Most probably all kinetoplastids have a plasma membrane that is divided into several functional domains. For example, in *T. cruzi,* axonemal, cytostomal field, and main body membrane differ in their lectin-binding capacities, sterol content, and appearance as seen in freeze-cleave preparations (De Souza, 1984). Each stage in the kinetoplastid life cycle may have a variety of glycoproteins and glycolipids exposed on its surface, some of which are stage specific and may vary in their structure from stock to stock. Differences between the surface membranes of several stages have been revealed by lectin-binding, complement activation, and measurements of surface charge. Reliable plasma membrane markers have not yet been identified in kinetoplastids. Much current research focuses on the function of exposed macromolecules as ligands binding to host cells during invasion, as antigens playing a part in the induction of

immunity and evasion of the host's immune response, or as receptors that bind external ligands to trigger off differentiation (Nogueira, 1983).

Among the interesting features of membrane lipid composition is the fact that cholesterol is incorporated if present in the surrounding medium, but these trypanosomatids are capable of ergosterol synthesis in its absence (Dixon *et al.*, 1971). Phosphatidylcholine and phosphotidylethanolamine are the most common phosphoglycerides in trypanosomatids.

The aspect of the trypanosome surface that has attracted most attention in recent years is the glycoprotein surface coat (Fig. 9) of the African trypanosomes (Vickerman, 1969) in relation to antigenic variation and its genetic control (Vickerman, 1978b; Borst and Cross, 1982; Turner, 1984; Boothroyd, 1985). The chronic infection and fluctuating parasitemia of African trypanosomiasis are due to evasion of the host's immune response through antigenic variation on the part of the parasite. Covering the entire trypanosome plasma membrane is a 12–15 nm thick coat composed of a monomolecular layer of a single species of glycoprotein. The antigenic nature of this glycoprotein can be changed repeatedly; each variant surface glycoprotein (VSG) is associated with a serologically identifiable variable antigen type (VAT). As trypanosomes multiply by fission in the ascending parasitemia, a particular VAT, the homotype, forms the major part of the population. When the host mounts an antibody (IgM) response to the homotype, the parasitemia goes into remission as trypanosomes of the homotype VAT are killed off. Immunofluorescence tests show that in addition to the homotype, however, a particular population contains minor VATs or heterotypes. These continue to multiply during remission and one of the heterotypes overgrows the rest to form the homotype of the next parasitemic peak (Van Meirvenne *et al.*, 1975). Heterotypes arise spontaneously in clonal populations of trypanosomes through the trypanosome's switching to expression of a different variable antigen gene. VAT switching does not require host antibody to induce it; the antibody simply plays a selective part in destroying the homotype in each parasitemic wave. Homotype VATs tend to appear in a characteristic sequence in the blood of an infected mammal, certain VATs being "predominant" and always arising early in the infection.

The total number of VATs that a trypanosome clone can produce is between 10^2 and 10^3. Each clone appears to have a characteristic repertoire of VATs and, within each parasite species, serodemes can be recognized, each serodeme being characterized by its VAT repertoire and corresponding to a particular trypanosome genotype. There is a single gene for each VSG. The glycoprotein is a single polypeptide chain with an exposed N-terminal portion and an unexposed C-terminus attached to the membrane proper (lipid bilayer). The various VSGs differ in the amino acid sequence of the N-terminus and hence in their antigenic specificity; the unexposed C-terminus contains common amino acid sequences. Carbohydrate groups are attached mostly to the C-terminus and in trypanosomes of the subgenus *Trypanozoon* are not exposed, so play no part in determining VAT specificity (reviewed by Turner, 1982). In *T. vivax* and *T. congolense*, lectin studies suggest that some of the carbohydrate groups may be exposed.

When the trypanosome embarks on cyclical development in the tsetse fly, the coat is discarded so the procyclic forms are uncoated. The coat is reacquired by the metacyclic trypanosomes in the vector's salivary glands (*T. brucei*) or hypopharynx (*T. vivax, T. congolense*). Uncoated *T. brucei*, noninfective to the mammal, activates complement by the alternate pathway and is readily phagocytosed by macrophages; coated stages are phagocytosed only in the presence of VAT-specific antibody, so the coat protects the parasite from the nonspecific defenses of its host (Vickerman and Barry, 1982). When the trypanosomes reacquire their coat at the metacyclic stage, each serodeme expresses a characteristic restricted repertoire of metacyclic VATs (M-VATs) that arise simultaneously as coating occurs. These M-VATs are the first to appear in the mammal following infected tsetse bite and are succeeded by the VAT originally ingested by the fly (I-VAT) and by the characteristic predominant VATs (Hajduk and Vickerman, 1981). The genetic basis of VAT expression is outlined below.

Because protective immunity to African trypanosomes is VAT-specific, vaccination of susceptible hosts against the full VAT repertoires of all local serodemes is a hopeless proposition. The most promising scheme is to confer protection against the M-VATS of local serodemes in the hope of suppressing infection at its inception (Vickerman and Barry, 1982).

Nutrition and Symbionts

Knowledge of nutritional requirements is fragmentary and difficult to summarize. In bodonids, a cytostome-cytopharynx is invariably present, and through it bacteria and macromolecules are endocytosed for digestion in food vacuoles. The preoral crest (Fig. 3a) and wall of the flagellar pocket form seizing lips for prey and these lips may be drawn out to form a prehensile rostrum (Fig. 1). In trypanosomatids, a cytostome-cytopharynx has been reported in *Crithidia* and certain invertebrate stages of the life cycle of some species of *Trypanosoma* (e.g., *T. cruzi, T. cyclops*). Pinocytosis of proteins can occur through this complex or directly from the membrane lining the pocket (Vickerman and Preston, 1976). Alternatively, membrane-mediated mechanisms may transport nutrients into the cell. In the majority of species, however, feeding mechanisms remain a mystery.

Lysosomes have been identified cytochemically in several trypanosomatids (Vickerman and Preston, 1976)—especially in those involved in active pinocytosis—but characterization with respect to enzyme content has been less frequent. In *T. brucei*, acid phosphatase, a common marker enzyme, is found mainly in the flagellar pocket and ER but not in organelles containing α-mannosidase, RNAase, phospholipase A, and an acid proteinase—suggesting lysosomal heterogeneity (Steiger *et al.*, 1980). A role has been suggested for lysosomes in drug uptake as well as in pathogenesis (Fig. 6).

Few trypanosomatids have been grown in defined media but these include representatives of the major genera. Actual nutritional requirements, however, have been determined for very few (e.g., *Crithidia fasciculata* and *"Leishmania tarentolae"* and species like *Crithidia* (=*Strigomonas*) *oncopelti*, which

possess bacterial endosymbionts, the symbionts providing a range of metabolites necessary for the growth of other kinetoplastids). The actual identity of *L. tarentolae* is open to dispute: recent evidence suggests that the culture promastigotes of this much-studied domesticated species may be a stage in the life cycle of *Trypanosoma platydactyli* and not a lizard leishmania (Wallbanks *et al.*, 1985). Trypanosomatids studied require minimally the following nutrients: at least 10 but often more amino acids, depending on other constituents of the medium; hemin; a purine (hemoflagellates can synthesize their own pyrimidines); thiamine, riboflavin, pantothenic acid, nicotinamide pyridoxamine, biotin, folic acid and biopterin; a mixture of inorganic nutrients and possibly specific lipids (Trager, 1974). The requirement of an exogenous source of unconjugated pteridine for growth may be unique to hemoflagellates and has led to the use of *C. fasciculata* as a biological assay for these compounds. A requirement for vitamin B_{12} has not been demonstrated (cf. euglenids).

Since they have no storage carbohydrates, kinetoplastids are primarily dependent upon exogenous substrates, such as carbohydrates or amino acids, for their energy supplies. All hemoflagellates can utilize glucose, fructose, and mannose; the ability to utilize disaccharides and pentoses is species- or even stock-dependent (see Newton, 1976b). Amino acids may also be utilized, however, and perhaps are, more commonly, in the insect gut. That the substrate may vary according to host in heteroxenous parasites was noted above. The amino acid requirements may differ in the presence or absence of carbohydrates as an energy source, e.g., *L. tarentolae* in a glucose-containing medium requires 10 amino acids for growth; in the absence of glucose, proline and glutamic acid are also required (Krassner and Flory, 1971). Amino acids as energy sources may be released from pinocytosed protein or by autophagy, which may account for the high endogenous rates of respiration observed in some hemoflagellates. Storage lipid occurs in several kinetoplastids, however, and the utilization of fatty acids as an energy source known to occur in *Leishmania* amastigotes (Hart and Coombs, 1982) may prove to be more widespread than is at present believed.

Symbiotic prokaryotes are common in the cytoplasm of bodonids and occur in some insect trypanosomatids; those of *Crithidia oncopelti, C. deanii* (of Hemiptera), and *Blastocrithidia culicis* have been studied in some detail. Symbionts relieve the former two (but not the last) species of their heme requirements; the bacterial symbionts also supply purines, eight amino acids, pyridoxine, riboflavin, and pantothenate to the trypanosomatids. When symbiont-free strains of these three species are produced by growth in the presence of chloramphenicol (Chang, 1975), their nutritional requirements become more complex. The kinetoplastid symbionts have not been cultivated free from the host cell. Those of bodonids appear to be intact encapsulated gram-negative bacteria (Eyden, 1977; Vickerman, 1977) whereas those of trypanosomatids appear to have lost the characteristic prokaryote cell wall and are hence regarded by some as organelles of possible symbiotic origin, in that case preferably referred to as "bipolar bodies" or "diplosomes" rather than symbionts. They obviously offer choice experimental material for students of organelle evolution.

Cytoskeleton

Microtubules are the only known element of the kinetoplastid cytoskeleton. Axonemal microtubules are involved in locomotion, pellicular microtubules (PMT) in the maintenance of cell shape, and nuclear spindle microtubules in cell division. In the bodonids the PMT array is incomplete (Brugerolle *et al.*, 1979) and composed of a series of microtubule bands ("fibers") as depicted for *Bodo caudatus* in Fig. 3a. In trypanosomatids, evenly spaced PMT form a corset enveloping the entire body (Figs. 4, 9); only in certain aposymbiotic insect trypanosomatids is the corset interrupted by PMT-aligned branches of the mitochondrion.

The major structural units, the α and β tubulin proteins, of trypanosomatid microtubules fall into three separate assembly-competent fractions (axonemal, PMT, and cytoplasmic tubulin pool). Two dimensional gel electrophoresis and peptide mapping show that the tubules of these fractions differ in molecular structure (Russell *et al.*, 1984), and monoclonal antibody studies have distinguished axonemal microtubules and PMT (Gallo and Anderton, 1983). There are multiple genes encoding for tubulin: the α and β tubulin genes of trypanosomatids are tandemly duplicated (Thomashow *et al.*, 1983; Seebeck *et al.* 1983); those of *Leishmania enriettii* are in two separate α and β tandem repeats, 15 copies in each. Whether the multiple tubulin genes of trypanosomatids give rise to different tubulin proteins remains to be investigated.

Microtubule assembly and disassembly play an important role in kinetoplastid morphogenesis and differentiation. There may be different regulatory mechanisms for α and β tubulin biosynthesis during development, and the leishmanias with the controllable amastigote > promastigote and promastigote > amastigote transformation provide favorable material for such studies (Fong *et al.*, 1984).

Motility Organelles and Movement

All kinetoplastids have two kinetosomes. Both bear flagella in the heterokont bodonids (Fig. 3). In the trypanosomatids the single axoneme-bearing kinetosome (Fig. 4) corresponds to the anterior flagellum of the bodonids. Attachment of the recurrent flagellum to the body occurs in several bodonids (Table 1) and in some trypanosomatid genera epimastigote and trypomastigote stages (Table 2). Nontubular mastigonemes are found on the anterior flagellum of certain bodonids (e.g., *Bodo saltans, Cephalothamnium cyclopum*). A linear series of desmosome-like attachments (Fig. 9) binds the flagellum to the body in trypanosomatids, but similar structures are rare in bodonids.

The axoneme of kinetoplastids (Fig. 9) has a characteristic "9 + 0" transition zone between it and the kinetosome, the two central axonemal microtubules arising beyond the second of two transverse plates delimiting the zone (Fig. 4). No arms are present on the doublets of the transition zone, and a fine "collarette" of unknown composition surrounds the zone out-

side its bounding membrane. A latticelike chord, the paraxial or paraflagellar rod running alongside the axoneme (Fig. 9), is a second characteristic of the kinetoplastid flagellum. The functional significance of these peculiarities is unknown. Striated rootlets are undeveloped in the kinetoplastids; the kinetosomes, which serve as microtubule organizing centers (Vickerman and Preston, 1976), are associated with the kinetoplast (mitochondrial) capsule membrane (Figs. 6, 8).

No detailed studies of movement in bodonids are available, but trypanosomatids have found a use in studies on hydrodynamic aspects of flagellar movement. A remarkable feature of these flagella is that they propagate waves from tip to base as well as from base to tip. Indeed, bends can be initiated at any point along the flagellum whereas in most other flagella bending is dependent upon activation in an adjacent region. Whether this property is related to the septum dividing the B microtubule (Fig. 9) of the axonemal doublet or any of the peculiarities mentioned above is uncertain (for a more detailed discussion see Holwill, 1980).

A final peculiarity of the kinetoplastid flagellum is its ability to act as an attachment organelle in parasitic species, securing the "haptomonad" to its substratum or to the flagellum of an adjacent kinetoplastid. The flagellum may develop extensive cortical outgrowths to aid attachment (Molyneux, 1983; Tetley and Vickerman, 1985). Such attached stages appear to play an important part in the life cycles of trypanosomatids. The physical nature of the attachment is unknown but hemidesmosome-like plaques are present on the axonemal membrane in attachments to a foreign substratum and desmosomes are present in attachments to other flagella (Vickerman, 1973; Molyneux, 1983).

Nucleus and Transcription; Sex and Genetics

All kinetoplastids have a vesicular nucleus with large central nucleolus (Fig. 6). Perinuclear chromatin is visible in electron micrographs (Fig. 6), but no chromosomal condensation cycle is apparent. Nuclear division takes place without dissolution of the nuclear envelope; an intranuclear microtubular spindle structure is present but lacks polar structures; a few kinetochorelike plaques which split into hemiplaques that migrate to the poles have been demonstrated (Solari, 1983). Evidence from quantitative absorption and fluorescence cytophotometry giving the amount of DNA per unreplicated cell, and from DNA-DNA association experiments giving the haploid DNA content, suggests that bloodstream and procyclic *T. brucei* are diploid (Borst *et al.*, 1982); trypanosomes might therefore be expected to have a sexual process in their life cycle. In studies on the isoenzymes of different *T. brucei* stocks, apparent hybrids for dimeric enzymes have been demonstrated and the frequency of homozygotes and heterozygotes conforms to the Hardy-Weinberg equilibrium, suggesting that the hybrids are the result of sexual processes (Tait, 1983). Hybrid formation in *T. brucei* has now been experimentally demonstrated following tsetse transimission of mixed parental stocks differing in isoenzyme and gene restriction fragment markers (Jenni *et al.*, 1986). Similar evidence has not been forthcoming for *T. cruzi*, but independent evidence from drug-resistant recombinant phenotypes suggests genetic recombination in *Crithidia fasciculata in vitro* (Glassberg *et al.*, 1985).

Although *T. brucei* appears to be diploid for "housekeeping" genes (e.g., those for glycolytic enzymes or tubulins), the variable antigen (VSG) genes may be present as single copies; it is possible that different VSG genes are present in corresponding positions of paired chromosomes (Gibson *et al.*, 1985). The number of copies of a given VSG gene in a nucleus may vary, however, and this may depend on whether or not that gene is being expressed.

Variable antigen type switching is often associated with transposition of the VSG gene for the new antigen to an expression site at the end of a chromosome. The "basic copy" of the gene duplicates and the copy moves to the telomeric site. This "expression-linked copy" (ELC) and the basic copy can be distinguished as separate bands in hybridization experiments in which an isotopically labelled cDNA copy of the gene (manufactured from the relevant messenger RNA using reverse transcriptase) binds to electrophoresed restriction enzyme (DNAase) digests of trypanosome nuclear DNA when these are blotted from the gels onto nitrocellulose filters. The mRNA for the expressed VSG gene is transcribed from the ELC, not the basic copy (Borst and Cross, 1982; Steinert and Pays, 1985).

In addition to the ELC mechanism for VAT switching, some genes which are always telomeric are activated *in situ* without a duplicative event; such genes include those coding for the metacyclic VATs and "predominant VATs"—i.e., those that appear early in the infection. About half the VSG genes studied to date are in this nonduplication activated category, which suggests that trypanosomes may have hundreds of chromosomes in order to accommodate so many telomeric genes. Pulsed field gradient electrophoresis in agarose gels allows chromosome-sized DNA molecules of *T. brucei* to be separated (Van der Ploeg *et al.*, 1984a) into four classes: (1) large chromosomes that remain in or near the origin; (2) several 2,000 kb chromosomes; (3) several intermediate (200–700 kb) chromosomes, the size and number varying with the stock; (4) perhaps 100 minichromosomes of 50–150 kb, again depending on the stock. VSG genes occur in all four classes. Extension of this technique to other trypanosomatids suggests that most species have at least 20 chromosomes although *Crithidia fasciculata* and *T. vivax* have fewer (Van der Ploeg *et al.*, 1984b). The genomic complexity of *T. brucei* is estimated at 4×10^4 kb (Borst *et al.*, 1982), allowing for 20 chromosomes of average size 2,000 kb. Chromosomes of classes 3 and 4 are present only in *Trypanozoon* and *Nannomonas* species (Table 3), the latter having the smallest linear chromosomes (25–50 kb) of any known organism. *T.* (*T.*) *equiperdum* has only 10 minichromosomes. As *T. vivax* lacks minichromosomes altogether their presence cannot be essential for antigenic variation; they may have evolved to allow more versatile use of that process, e.g., by allowing for frequent recombination between telomeres giving rise to new VSGs (Steinert and Pays, 1985).

In general, Kinetoplastida, then, resemble fungi in having many small chromosomes rather than a few large ones, but

chromosome size and composition are not highly conserved within the order—suggesting a versatile genomic organization that allows rapid evolution.

An extraordinary feature of transcription in trypanosomatids is that it appears to be a discontinuous process. Most trypanosomatid mRNAs share a common 5′ terminal sequence of 35 nucleotides encoded by a "mini-exon" located in 1.35 kb tandemly-linked repeats, and often on a separate chromosome from the specific gene. It is concluded that production of the mature mRNA must involve splicing of transcripts separately generated by different RNA polymerases. Uniform leader sequences common to most, if not all, mRNAs are rare in other cells (De Lange *et al.*, 1984; Nelson *et al.*, 1984; Boothroyd, 1985).

Classification Schemes, Special Identification Procedures, and Unsolved Problems

An amplified and revised classification of the order Kinetoplastida was given by Vickerman (1976), who recognized three families rather than one in the Bodonina: the Bodonidae (*Bodo, Rhynchomonas, Ichthyobodo*), phagotrophic and with unattached recurrent flagellum; the Cryptobiidae (*Cryptobia, Dimastigella, Cephalothamnium*) with attached recurrent flagellum and either osmotrophic or phagotrophic feeding; and the Trypanophidae (*Trypanophis*) with attached recurrent flagellum in flagellar gutter, osmotrophic and with inconspicuous kinetoplast. The existence of varying degrees of recurrent flagellum attachment in the new phagotrophic genus *Procryptobia* (Vickerman, 1978a) suggests that separation of the first two families is artificial, and the absence of a recognizable kinetoplast and presence of multiple golgi systems in *Trypanophis* suggest that the last subfamily belongs only doubtfully in the Kinetoplastida. Hence only one family is recognized within the suborder Bodonina here.

The thorniest taxonomic problems lie in species recognition, especially among the trypanosomatids of practical importance. Here, exact identification is necessary for epidemiological studies, but morphology is of no help; biochemical approaches, however, have proved most useful. The cloning of stocks is essential for meaningful comparisons of the information content of genomes and of biochemical phenotypes. The broad problems have been discussed by Newton (1976b) and Gibson and Miles (1985). Three different technologies are currently being employed in epidemiological research. These are the use of isoenzymes as genetic markers, direct analysis of DNA, and the emergent use of highly specific monoclonal antibodies.

Isoenzyme bands can be assigned to particular genetic loci and alloenzyme frequencies used to calculate genetic distances. Dendrograms and cladograms constructed from genetic distances give a measure of taxonomic divergence. For epidemiological studies, isoenzyme data and other parameters can be incorporated into more complex, computer-based cluster analyses, and distinct population clusters may, as a result, become apparent. These can then be related to geographical, ecological, or clinical features. Conclusions of considerable epidemiological significance have emerged from such work (Gibson and Miles, 1985). (see also *Nucleus and Transcription*, p. 231 and Evolutionary History, p. 234).

The limited complexity and ease of purification of kDNA have been exploited for taxonomic purposes by electrophoretic comparison of kDNA fragments released by specific endonuclease digestion, using ethidium bromide fluorescence to localize the bands. Specific groups based on kDNA diversity are termed schizodemes. Particular kDNA fragments that are genus, species, or even strain specific can be isolated and radio-labeled to produce probes for the identification of unknown trypanosomes, or probes can be dye-labeled for field use. The advantage of this technique is that small numbers of organisms can be identified by the hybridization to specific probes on nitrocellulose or microscope slides. The technique has been used for rapid specific identification of *Leishmania* spp. in skin lesions (Wirth and Pratt, 1982), and has superseded less specific methods of DNA analysis such as buoyant density determination (Newton, 1976b).

Monoclonal antibodies that react with genus, species, or strain specific antigens can be manufactured and reactivity to a panel of such antibodies can be used to classify trypanosomatid populations. Such attempts at classification are still in their infancy, however.

Attempts to find reliable markers for man-infectivity and hence subspecies recognition in *T. brucei* have so far been unsuccessful. Although *T. b. gambiense* stocks can be distinguished by their isoenzyme profile, low virulence to rodents, and VAT repertoire (serodeme), distinction between *T. b. brucei* and *T. b. rhodesiense* still requires a test that avoids infection of human volunteers. Sensitivity of bloodstream trypanosomes to human serum has been widely used in this respect. *T. brucei* is reputedly lysed by a factor in normal serum, whereas *T. rhodesiense* is not. Such serum sensitivity of trypanosomes can appear and disappear during the course of a clone infection of *T. brucei* in laboratory animals. Thus, whether human serum sensitivity, or even human infectivity alone, is a satisfactory criterion on which to separate subspecies is highly dubious (Rickman *et al.*, 1984).

MAINTENANCE AND CULTIVATION

In Vivo Maintenance

Monoxenous trypanosomatids are nonpathogenic to their hosts and infected colonies of insects, for example, can be maintained. With uninfected ones, contaminative transmission of fresh hosts occurs naturally. Digenetic trypanosomatids are usually maintained by syringe passage of infected blood or tissue in the vertebrate host; such continuous mechanical transmission may result in loss of ability to infect the vector. Many species of *Trypanosoma* and *Leishmania* transfer readily to other related hosts. Most notorious of those that fail to transfer are the extremely host specific trypanosomes of the subgenus *Herpetosoma, T. vivax,* and *T. b. gambiense*. However, rodent-adapted lines of the last two species have been described (Leeflang *et al.*, 1976; Babiker and LeRay, 1981). Ionizing irradiation of the host has been used to increase yields of bloodstream salivarian trypanosomes and intracellular *T. cruzi* (Gutteridge *et al.*, 1978). Ion exchange chromatography has

proved most useful for separating trypanosomes from accompanying blood cells (Lanham, 1968). For separation of intracellular leishmanias and *T. cruzi* from host cells, see Hart *et al.* (1981) and Sanderson *et al.* (1980).

Cryopreservation of these organisms in infected blood with glycerine or dimethylsulfoxide as cryoprotectant (Lumsden, 1972) has greatly facilitated research, but care must be taken to obtain trypanosomatids from reliable laboratories as confusion of vials happens frequently.

Free-living Kinetoplastids

The phagotrophic free-living bodonids may be isolated in monoprotist culture from rich infusions of soil, feces, or vegetable matter by serial dilution with soil extract (SE) diluted 1:10 with distilled water (or sea water in the case of marine species). Monoxenic cultures can sometimes be obtained by migration. Packed, washed cells are layered on the surface of diluted SE medium in a long Pasteur pipette whose sealed fine end has been bent repeatedly to give a zig-zag path. Flagellates migrate toward the sealed tip leaving behind their accompanying bacteria, so that axenic specimens can be recovered from the tip and placed in 0.05% liver infusion with a suitable food bacterium (e.g., *Enterobacter aerogenes*); subculture at weekly intervals. Axenic cultures of these organisms have not been achieved.

Monoxenous Trypanosomatid Hemoflagellates

Initial cultivation of these Trypanosomatidae (often called "lower trypanosomatids") is usually on complex biphasic blood media (Evans, 1978) such as NNN, brain-heart infusion agar, or Diamond's SNB9 medium (this last utilizes autoclaved blood agar and is particularly suitable for use under field conditions). Widely used complex monophasic media are FYTS (Roitman *et al.*, 1972) and LIT medium (Carmargo, 1964). Bacterial contamination may be controlled with 250 μg/ml gentamycin. Yeast or other fungal contamination is best eliminated by use of a capillary migration tube, but 500 μg/ml 5-fluorocytosine may prove useful in primary cultures. Insect hosts in particular are liable to be infected with several species, or even genera, of trypanosomatids, so once a culture is isolated and growing vigorously, cloning should be attempted. Plating on blood agar (Tanuri *et al.*, 1981) is the preferred method for both monoxenous trypanosomatids and vector stages of the heteroxenous parasites. For plating and characterization of mutants see Glassberg *et al.* (1985).

For rapid cultivation of hemoflagellates in quantity, commercially available liquid tissue culture media are now routinely used. These include Medium 199, Grace's insect tissue culture medium, and Schneider's *Drosophila* medium (all Grand Island Biological Co., Long Island, New York). These media can be freeze dried and as such have a long shelf life. Before use 1–30% heat inactivated fetal calf serum must be added. More rapid and luxuriant growth is obtained in the insect media. Most monoxenous trypanosomatids, species of *Leishmania*, and some stercorarian trypanosomes can be cultured in these media (Hendricks *et al.*, 1978).

A defined medium widely used for several hemoflagellates has been described by Roitman *et al.* (1972). Symbiont-containing hemoflagellates may be cultured routinely on simple heme-lacking media (Chang *et al.*, 1975).

Heteroxenous Hemoflagellates

Only certain stages in the life cycles of heteroxenous hemoflagellates may be obtained in culture, usually those found naturally in the vector. Of the parasitic bodonids, only trypanoplasms of freshwater fish have been cultivated to date; both bloodstream and vector stages appeared in cultures on SNB9 medium (made with outdated human blood) subcultured at 14-day intervals; incubation temperature is critical (10°–20°C, Hajdu and Matskasi, 1984). *Cryptobia* (*Trypanoplasma*) *salmositica* has been cultivated from cardiac blood of salmon in 10 ml Hanks' TC solution (Difco) with 10% inactivated fetal calf serum, subculturing every 15-days; culture forms are infective to fish (Woo, 1979).

Leishmania species and trypanosomes of poikilothermic vertebrates may be isolated and grown on a variety of biphasic and monophasic media (see Evans, 1978). *Leishmania* grows with promastigote morphology. For isolation, biopsies of the lesion (or bone marrow aspirate in the case of *L. donovani*) are transferred to blood agar slopes with added antibiotics. When flagellates appear on the slopes, the techniques used are the same as those for subculture and further cultivation of monoxenous hemoflagellates. Culture promastigotes can be used to initiate the vertebrate host cycle in mammalian tissue culture systems, usually macrophage-derived cell lines (Berens and Marr, 1979; Chang, 1983). Growth of the amastigote stage outside the macrophage host cell in a complex medium has been reported by A.A. Pan (1984). Defined media for the growth of leishmania promastigote stages are available (Melo *et al.*, 1985).

The stercorarian trypanosomes vary in their ease of isolation *in vitro*; species of the subgenus *Megatrypanum* are the easiest. Those of the subgenus *Herpetosoma* are the most difficult to establish in the media described above for monoxenous hemoflagellates. Some success in cultivating mammalian stages in the life cycles of species of these two subgenera in conjunction with mammalian cells in culture has also been reported (Mansfield, 1977).

T. (*Schizotrypanum*) *cruzi* and related species may be easily grown as the epimastigote stage in LIT and a variety of other media, including semi-defined and defined ones (O'Daly, 1975). Use of a continuous flow system (Williams and Hudson, 1982) improves yields. Metacyclic trypanosomes are readily produced in these cultures and their yield can be increased by adjusting pH to 5.5 and use of Grace's medium (Sullivan, 1982). Various cell types can be used to grow the intracellular trophic amastigote stage, e.g., chick embryo, bovine embryo muscle, HeLa, Vero, myocardial cells (for refs., see Bioul-Marchand *et al.*, 1980); nondividing trypomastigotes are released into the medium and can be collected to infect fresh cultures. If high trypomastigote yields are required the procedures of Sanderson and co-workers (1980) may be followed. *T.*

cruzi amastigotes reportedly grow in a cell-free medium (S.C. Pan, 1978).

The salivarian trypanosomes (except *T. vivax*, which lacks a vector midgut stage in the life cycle) are readily grown in bulk as the vector midgut procyclic form. *T. brucei* procyclics can be produced in large quantities in complex, semi-defined, or, for some stocks, even a defined medium (for refs., see Brun and Jenni, 1985). The salivary gland stages are difficult to produce *in vitro*; cultivation of procyclics in the presence of tsetse head-salivary gland or pupal tissue explants (Cunningham *et al.*, 1981) results in the generation of up to 0.3% metacyclics; a simple system is not available at present, the requirement for metacyclogenesis of attachment to gland epithelium proving a difficult problem to overcome. The blood and extravascular tissue forms of *T. brucei* and related species were first cultivated by Hirumi and co-workers (1977) using bovine fibroblast cultures as feeder layers. Since then several cell lines as well as primary murine bone marrow cells (Balber, 1983) have been shown to support continuous growth of bloodstream forms. The medium used is RPMI 1640 or Eagles MEM with Earle's salts, both media supplemented with 25 mM HEPES buffer. More recently cultivation of bloodstream forms of *T. brucei* (including both sleeping sickness subspecies), *T. evansi*, and *T. equiperdum* has been achieved in a semi-defined medium without a feeder cell layer (Baltz *et al.*, 1985). Supplements of 0.2 mM 2-mercaptoethanol, 2 mM pyruvate, and 10% inactivated rabbit or human (for *T. gambiense*) serum enabled the feeder cells to be dispensed with, although a macrophage feeder layer is recommended for primary isolation of trypanosomes from the mammalian host. It is believed that feeder cell layers eliminate toxic free radicals from the cultures.

T. congolense procyclics can be obtained in primary culture (from host blood) and grown in bulk in a semi-defined medium (Brun, 1982). The tsetse proboscis stages (epimastigote—metacyclic) of this species have been cultivated by transferring infected tsetse probosces into a semi-defined medium alongside bovine dermal collagen explants (Gray *et al.*, 1981). Serial subculture does not require the explant. Bloodstream stages of this species have now been grown in continuous culture on a bovine endothelial cell layer in RPMI with goat serum supplement (Hirumi and Hirumi, 1984).

Stages in the life cycle of *T. vivax* have been cultivated by introducing bloodstream forms into bovine fibroblast culture at 25°C along with Matrix Green gel beads. The attached vector proboscis phase occurs on beads and, later, feeder cells; a small proportion of metacyclic forms are produced. Bloodstream *T. vivax* (West African) has recently been cultured with *Microtus* embryo fibroblasts and 20% goat serum in the overlay (Brun and Jenni, 1985).

Population doubling times vary from 6 hours to 18 hours in trypanosomatid cultures and yields of up to 10^7 organisms/ml can be obtained under optimum growth conditions.

EVOLUTIONARY HISTORY

Biochemical evidence based on cytochrome (*b,c*) amino acid sequence data and 18S small ribosomal subunit nucleotide sequence data suggests that the Kinetoplastida separated from other eukaryote groups approximately one billion years ago, i.e., shortly after the appearance of eukaryotes on earth (Opperdoes, 1985).

There are no fossil kinetoplastids and so the evolutionary history of the group, a matter for conjecture only, must be reconstructed from comparative morphology and biochemistry. The euglenids, which have more morphological features in common with kinetoplastids than any other extant group (basically two flagella with paraxial rods and flagellar pocket, peripheral microtubular cytoskeleton, mitochondrial network with discoid cristae, nuclear division with persistent nucleolus), may have provided an ancestor for the group. But whereas euglenids, like fungi, synthesize lysine by the α-aminoadipic acid route, those kinetoplastids that can synthesize lysine do so by the diaminopimelic acid route. Massive development of the mitochondrial DNA to form the kinetoplast sets the group apart, as does segregation of enzymes of the glycolytic chain in the glycosome.

The biflagellate bodonids are likely to have evolved earlier than the uniflagellate trypanosomatids, because the former group contains both free-living and parasitic forms. *Bodo* itself is probably closest to the ancestral kinetoplastid condition. Development of the rostrum for prey capture (*Rhynchomonas*) and eventually histophagy (*Ichthyobodo*) may have been one evolutionary line; in a second line, attachment of the recurrent flagellum (*Dimastigella, Procryptobia, Cephalothamnium*) possibly paved the way for propulsion in more viscous media and invasion of a variety of hosts with the adoption of osmotrophy in *Cryptobia*. The digenetic trypanoplasms probably represent the most recently evolved bodonids. A third evolutionary line may have led to the trypanosomatids.

Loss of the recurrent flagellum, leaving only the anterior as the organ of propulsion, gave rise to the trypanosomatids; and catenation of the circular kDNA molecules to form a network may have occurred at the same time. The promastigote *Leptomonas* genus represents the simplest form, and *Crithidia* and *Herpetomonas* possibly represent later development in one-host parasitism. In a second lineage, *Phytomonas* and *Leishmania* retain the simple promastigote form while becoming two-host parasites. Baker (1974) suggests that *Endotrypanum* and the subgenus *Schizotrypanum* are later developments of this same line since they retain promastigote stages in the life cycle. He derives the rest of the genus *Trypanosoma* from a third trypanosomatid stock in which the epimastigote form has replaced the promastigote, *Blastocrithidia* and *Rhynchoidomonas* being possible monogenetic progenitors. The hematozoic habit (living in animal blood) evolved in at least three separate lineages: in the cryptobia stock of the bodonids and in the promastigote and epimastigote stocks of the trypanosomatids; in the trypanosomatids when the insect host became hematophagous; and in the trypanoplasms when the blood-gills-skin contact route of transmission was improved by leech transmission. The insect-borne trypanosomes of amphibia and reptiles (Bardsley and Harmsen, 1973) are thought to have arisen from leech-transmitted forms as these vertebrates became more terrestrial in their life style.

These ideas are based on comparisons of parasite morphology, life cycles, and host behavior and evolution. The evolutionary implications of studies on kDNA and antigenic variation are discussed in Characterization and Recognition (p. 225).

Only in the salivarian trypanosomes of mammals, however, can a convincing evolutionary pathway be reconstructed with any degree of confidence (Hoare, 1972). The increasing complexity of the vector cycle in *Glossina* from *T.* (*Duttonella*) *vivax*, through *T.* (*Nannomonas*) *congolense* to *T.* (*Trypanozoon*) *brucei* can be seen as a progression. *T. congolense* and *T. brucei* have interpolated in the life cycle a midgut development phase (absent from the *T. vivax* cycle), while *T. brucei* shows an advance over the other two species in that the final multiplicative phase and metacyclogenesis occur in the tsetse fly's salivary glands rather than in its proboscis. Within the subgenus *Trypanozoon* itself, recent isoenzyme studies have corroborated Hoare's view that *T. evansi* evolved from the cyclically transmitted *T. brucei* of the African tsetse belt by transportation across the Sahara in camel trains. Tabanid flies served to transmit the blood infection mechanically with concomitant loss of trypanosome pleiomorphism. From North Africa, *T. evansi* has spread in horses east across Southern Asia to the East Indies and Taiwan, and west to South America. In the latter case, vampire bats as well as biting flies have become responsible for transmission. A further development in transmission was the spread of *T. evansi* by contact between horses in coition, giving rise to the venerally transmitted *T. equiperdum*. Wider speculation has invoked the devastatingly lethal effect of trypanosome infections on native mammal populations to explain the extinction of the graviportal ungulates of South America after the artiodactyls and perissodactyls invaded that continent at the end of the Pliocene. Indeed one wonders if the fifth plague of the Egyptians in biblical times, when the murrain of the animals followed a plague of biting flies, was attributable to the kinetoplastid, *T. evansi!*

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